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(71) Applicant *(for all designated States except US)*: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YROK [US/US]; 116th Street & Broadway, New York, NY 10027 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ERLANGER, Bernard, F. [US/US]; 163-16 15th Drive, Whitestone, NY 11357 (US). LEU, Jyh-Gang [US/US]; Apartment 2L, 542 West 112th Street, New York, NY 10025 (US). CHEN, Bi-Xing [US/US]; Apartment 2B, 4 South Pinehurst Avenue, New York, NY 10033 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham, 30 Rockfeller Plaza, New York, NY 10112 (US).

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(54) Title: AN ENDOGENOUS TAXOL-LIKE SUBSTANCE IN HUMAN SERUM, MONOCLONAL ANTIBODIES DIRECTED THERETO, AND METHODS OF ASSAYING THEREFOR

(57) Abstract

The present invention provides two monoclonal antibodies capable of binding to taxol and taxol-like substances which are produced by hybridomas designated 69E4A8E, having ATCC Accession No. HB 11281 and 29B7B3C, having ATCC Accession No. HB 11280. The present invention also provides a method for detecting the presence of taxol or a taxol-like substance in a sample, a method for screening for a ligand in a subject which is not being treated with taxol, an endogenous taxol-like substance in human serum detected by this screening method, a method of quantitatively determining the amount of taxol or taxol-like substance in a biological fluid sample and a kit for assaying for taxol or a taxol-like substance in a sample. The present invention further provides an anti-idiotypic monoclonal antibody which mimics taxol. The invention provides an anti-idiotypic monoclonal antibody produced by hybridoma designated 82H11B9F, having ATCC Accession No. HB 11548. The present invention also provides methods of using the anti-idiotypic monoclonal antibody for determining the presence and amount of taxol or biologically active taxol derivatives and the presence and amount of receptors for taxol or biologically active taxol derivatives.

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AN ENDOGENOUS TAXOL-LIKE SUBSTANCE IN HUMAN SERUM, MONOCLONAL ANTIBODIES DIRECTED THERETO, AND METHODS OF ASSAYING THEREFOR

This application is a continuation-in-part of U.S. Serial No. 08/025,557, filed March 3, 1993, the contents of which are hereby incorporated by reference. The invention described herein was made in the course of work under Grant No. 1 R55 CA-55159 from the National Institutes of Health. The U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various publications are referenced by Arabic numerals in parentheses. Full text citations of these publications can be found at the end of the specification, immediately preceding the claims. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Taxol, a compound extracted from the western yew, Taxus brevifolia, is a diterpenoid, which has a 20 carbon, skeleton, with a complex ester side chain at C-13 and an oxetane ring. Taxol was shown to have antitumor activity (1). Its underlying mechanism is to promote and stabilize microtubule assembly and inhibit disassembly to tubulin (2). The binding site of taxol in microtubules differs from that of other anti-tublin drugs, such as colchicine, podophyllotoxin and vinblastine, which inhibit tubulin polymerization (3, 4).

In clinical trials, taxol was found to be affective in the treatment of ovarian (5, 6) and breast cancer (7) and melanoma (8). As with all anti-cancer agents, there are

side effects, in this case neutropenia, hypersensitivity reactions mucositis, neurological and possible cardiac toxicity were reported during clinical trials (9). It would be useful, therefore, to be able to measure taxol levels in patient under treatment in order to optimize treatment. A sensitive assay for measuring taxol levels is by HPLC (9, 10). In this application, we describe a polyclonal and two monoclonal antibodies that can be used to measure taxol levels in human serum with high sensitivity and is more amenable for the measurement of large numbers of samples. The monoclonal antibodies also have the potential to be used to screen for taxol or taxol-like substances in extracts of natural products.

The three dimensional structure of taxol has an inverted cup-like shape. Gueritte-Voegelein et al. (16, 18) determined the three dimensional structure of taxotere, a semi-synthetic biologically active taxol analogue, by X-ray analysis; it also has an inverted cup shape and the same skeleton as taxol. The taxotere molecule is stabilized by intramolecular hydrogen bonds between C-3'H and the C-4 acetyl group and between C-2'H and C-18H_3, as well as a repulsive interaction between the substituents at C-'2, C-3' and the taxane skeleton (16).

Structure-activity studies have revealed that the C-13 ester side chain (19, 20) and a closed oxetane ring (10, 21) are crucial to the activity of taxol derivatives. Opening of the oxetane ring results in considerable conformational change of the molecule (10, 21). Modification of substituents at C-10 and/or C-17 can alter activity but not markedly (10, 19, 20).

This application describes three antibodies specific for taxol: one rabbit antiserum and two monoclonal antibodies. With respect to the monoclonal antibodies, one is an IgG (69E4A8E) and the other an IgM (29B7B3C).

All of them bind taxol and active derivatives well and can be used to measure taxol levels in human serum.

Summary of the Invention

The present invention provides a monoclonal antibody which is capable of binding to taxol and taxol-like substances and is produced by a hybridoma cell designated 69E4A8E, having ATCC Accession No. HB 11281.

The present invention also provides a second monoclonal antibody capable of binding to taxol or taxol-like substances produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

The present invention also provides a method for detecting the presence of taxol or a taxol-like substance in a sample which comprises treating the sample with either of the above-identified monoclonal antibodies permitting the taxol or taxol-like substance in the sample to bind to the monoclonal antibody, removing antibodies which did not bind to taxol or the taxol-like substance, and detecting the presence of any bound antibodies, thereby detecting the presence of taxol or the taxol-like substance in the sample.

The present invention also provides a method for detecting the presence of taxol or a taxol-like substance in a sample which comprises contacting a predetermined amount of taxol or taxol-like substance labeled with a detectable marker with either of the above-identified monoclonal antibodies under conditions permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance, contacting the sample with the complex such that any taxol or taxol-like substance in the sample will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody, separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance, and detecting the presence

of any bound, detectably labeled taxol or taxol-like substance, thereby detecting the presence of taxol or taxol-like substance in the sample.

The present invention further provides a method for screening for a ligand in a subject which is not being treated with taxol which comprises obtaining a sample from a subject, contacting the sample with either of the above-identified monoclonal antibodies permitting the taxol or taxol-like substance in the sample to bind to the monoclonal antibody, removing antibodies which did not bind to taxol or the taxol-like substance, and detecting the presence of any bound antibodies in the sample, thereby detecting the presence of taxol or the taxol-like substance in the subject.

The present invention also provides an endogenous taxol-like substance in human serum which can be detected by the above-identified method.

The present invention further provides a method of quantitatively determining the amount of taxol or taxol-like substance in a biological fluid sample which comprises contacting a solid support with an excess of a composition of matter comprising taxol and an appropriate carrier molecule, contacting a predetermined amount of a biological fluid sample and a predetermined amount of either of the above-identified monoclonal antibodies permitting the taxol or taxol-like substance in the sample to bind to the monoclonal antibody, contacting the solid support to which the composition of matter is attached with the solution permitting antibodies which do not form a complex with the taxol or taxol-like substance in the biological fluid sample to bind to the composition of matter, treating the solid support so that only the composition of matter and monoclonal antibody bound thereto remain, and determining the amount of antibody

bound to the composition of matter, thereby determining the concentration of taxol or taxol-like substance in the biological fluid sample.

The present invention further provides a method of quantitatively determining the amount of taxol or taxol-like substance in a sample which comprises contacting a predetermined amount of detectably labeled taxol or taxol-like substance with either of the above-identified monoclonal antibodies under conditions permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance, contacting the sample with the complex under appropriate conditions such that any taxol or taxol-like substance in the sample will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody, separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance, and determining the amount of bound, detectably labeled taxol or taxol-like substance, thereby determining the amount of taxol or taxol-like substance in the sample.

The present invention provides a kit for assaying for taxol or a taxol-like substance in a sample comprising in separate compartments either of the above-identified monoclonal antibodies, a second monoclonal antibody which is labeled with a detectable marker and is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance, and a standardized solution of taxol.

The present invention provides a method for the detection of taxol or taxol-like substances present in biological fluids during treatment with taxol or taxol like substances using the above-identified kit.

The present invention also provides an anti-idiotypic monoclonal antibody of taxol or biologically active taxol derivative.

The present invention further provides a hybridoma which produces the above defined anti-idiotypic monoclonal antibody.

The present invention provides a hybridoma designated 82H11B9F and having ATCC Accession No. HB 11548.

The present invention provides a method of detecting taxol receptors in a sample which comprises contacting the sample with the above defined anti-idiotypic monoclonal antibody under conditions such that the antibody forms a complex with a receptor and detecting the presence of any complex so formed.

The present invention also provides a qualitative immunoassay for detecting a taxol receptor which comprises contacting a sample suspected of containing the taxol receptor with the above defined anti-idiotypic monoclonal antibody so as to form a complex which includes the detectably labeled anti-idiotypic monoclonal antibody and the taxol receptor; and detecting the presence of the complex and thereby detecting the presence of the taxol receptor.

The present invention provides a qualitative histochemical assay for detecting the presence of a taxol receptor in a biological sample which comprises incubating the biological sample with the above defined anti-idiotypic monoclonal antibody so as to form a complex which includes the taxol receptor and the anti-idiotypic monoclonal antibody; removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex; contacting the resulting sample with a

detectably labeled antibody or detectably labeled antibody fragment capable of specifically binding the anti-idiotypic monoclonal antibody under conditions permitting the labeled antibody or antibody fragment to bind to the anti-idiotypic monoclonal antibody; and determining the presence of detectably labeled antibody fragment bound to the anti-idiotypic monoclonal antibody and, thereby, detecting the presence of taxol receptor in the sample.

The present invention further provides a quantitative histochemical assay for determining the amount of a taxol receptor is present in a biological sample which comprises treating the biological sample with a predetermined amount of the above defined anti-idiotypic monoclonal antibody to form a complex which includes the taxol receptor and the anti-idiotypic monoclonal antibody; removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex; contacting the resulting sample with a detectably labeled antibody or a detectably labeled antibody fragment capable of specifically binding to the anti-idiotypic monoclonal antibody under conditions permitting the detectably labeled antibody or detectably labeled antibody fragment to bind to the anti-idiotypic monoclonal antibody which is part of the complex formed; and determining the amount of detectably labeled antibody or detectably labeled antibody fragment bound to the anti-idiotypic monoclonal antibody and thereby determining the amount of taxol receptor in the sample.

The present invention also provides a method for determining the amount of taxol or biologically active taxol derivative in a sample which comprises immobilizing the above defined anti-idiotypic monoclonal antibody on an appropriate substrate; contacting the sample containing taxol or biologically active taxol derivative

with the immobilized anti-idiotypic monoclonal antibody under conditions permitting taxol or biologically active taxol derivatives in the sample to form a complex with the immobilized anti-idiotypic monoclonal antibody; contacting the complex formed with a predetermined amount of a labeled antibody or labeled antibody fragment which specifically binds to taxol or biologically active taxol derivative under conditions permitting the labeled antibody or labeled antibody fragment to displace taxol or biologically active taxol derivative bound to the immobilized anti-idiotypic monoclonal antibody; and determining the amount of labeled antibody or labeled antibody fragment bound to the immobilized monoclonal anti-idiotypic monoclonal antibody thereby determining the amount of taxol or biologically active taxol in the sample.

Finally, the present invention provides method for determining the amount of taxol or biologically active taxol derivative in a sample which comprises immobilizing an antibody or antibody fragment which specifically binds to taxol or biologically active taxol derivatives on an appropriate substrate; contacting the sample containing taxol or biologically active taxol derivative with the immobilized antibody or antibody fragment under conditions permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized antibody or antibody fragment; contacting the complex formed with a predetermined amount of the above defined anti-idiotypic monoclonal antibody labeled with a detectable marker under conditions permitting the labeled anti-idiotypic monoclonal antibody to displace any taxol or biologically active taxol derivative bound to the immobilized antibody or antibody fragment; and determining the amount of labeled anti-idiotypic monoclonal antibody bound to the immobilized antibody or antibody fragment thereby determining the amount of taxol

or biologically active taxol derivative in the sample.

Brief Description of Figures

Figure 1. Inhibition of the binding of rabbit anti-taxol antiserum to [^{3}H]taxol by taxol derivatives and analogues. o, taxol; ●, cephalomannine; ■, taxotere; ▲, baccatin III; X, 20-acetoxy-4-deacetyl-5-epi-20, O-secotaxol. The results are means of duplicate points and are expressed as the percentage of inhibition relative to the specific [^{3}H]taxol binding measured in the absence of inhibitors in RIA.

Figure 2. Inhibition of the binding of 29B7B3C to taxol-RSA-coated wells by taxol derivatives and analogues. o, taxol; ●, cephalomannine; □, 7-epitaxol; ■, taxotere; Δ, 2'-(triethylsilyl)taxol; ▲, baccatin III; X, 20-acetoxy-4-deacetyl-5-epi-20, O-secotaxol. The results are means of duplicate points and are expressed as the percentage of inhibition relative to the absorbance at 490 nm measured in the absence of inhibitors in the ELISA.

Figure 3. Inhibition of the binding of 69E4A8E to taxol-RSA-coated wells by taxol derivatives and analogues. o, taxol; ●, cephalomannine; □, 7-epitaxol; ■, taxotere; Δ, 2'-(triethylsilyl)taxol; X, 20-acetoxy-4-deacetyl-5-epi-20, O-secotaxol. The results are means of duplicate points and are expressed as the percentage of inhibition relative to the absorbance at 490 nm measured in the absence of inhibitors in the ELISA assay.

Figure 4. Inhibition of binding of 82H11B9F to Fab' of affinity pruified rabbit anti-taxol by taxol or taxol derivatives: ●, taxol; o, taxotere; ■, baccatin III.

Figures 5A and 5B. Inhibition of binding of [^{3}H]taxol to microtubules. Figure 5A: Inhibition of binding of [^{3}H]taxol to microtubules by taxol or taxol analogues: ●, taxol; o, taxotere; ■, baccatin III. Figure 5B:

Inhibition of binding of [^{3}H]taxol to microtubules by anti-idiotypic monoclonal antibodies 82H11B9F (-●-) and 26F (-o-), an anti-thymidine antibody.

Figure 6. Polymerization of microtubules by different dilutions of anti-idiotypic monoclonal antibody 82H11B9F: 6.5 μM (-●-) and 3.25 μM (-o-). The turbidimetric assay of Parness et al. (19) was used except that the antibody or taxol, in 480 ul of PBS + 0.1% NaN_3, was added to tubulin in 620 ul of MES buffer. The final pH was 6.75.

Figures 7A-7C. Electron microscopic examination of the suspension produced in experiments described in Figure 6. A drop of the suspension produced by the action of 82H11B9F on tubulin was put onto a grid and allowed to remain there for 15 seconds. Excess fluid was withdrawn using a filter paper wick, and one drop of 1% uranyl acetate was added to the grid. Excess uranylacetate was immediately drawn off with filter paper, the grid allowed to air dry for several hour at room temperature and examined with a electron microscope. Figure 7A: magnification of 10,000; Figure 7B: magnification of 40,000; Figure 7C: magnification of 200,000.

Detailed description of the invention

The present invention provides two monoclonal antibodies capable of binding to taxol or taxol-like substances. In one embodiment the monoclonal antibody is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

In one embodiment of the invention the monoclonal antibodies are capable of binding to taxol-like substances which are biologically active taxol derivatives. Those derivatives of taxol which are presently known to be biologically active are well known to those skilled in the art. At the time of this invention structure-activity studies have revealed that the C-13 ester side chain (19,20) and a closed oxetane ring (10,21) are crucial to the activity of taxol derivatives. In a preferred embodiment of this invention the monoclonal antibodies are capable of binding to biologically active taxol derivatives. It is anticipated that the monoclonal antibodies of the present invention will bind to any taxol-like substances presently known or later discovered, including later discovered biologically active taxol derivatives or taxol-like substances.

The present invention also provides hybridomas which produce the monoclonal antibodies capable of binding to taxol or taxol-like substances and are designated 69E4A8E (ATCC Accession No. HB 11281) and 29B7B3C (ATCC Accession No. HB 11280).

In one embodiment of this invention the monoclonal antibodies produced by the above-identified hybridomas are labeled with a detectable marker. Detectable markers

useful for detecting monoclonal antibodies are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In one preferred embodiment the monoclonal antibodies are labeled with a radioactive isotope. In a second preferred embodiment the monoclonal antibodies are labeled with an enzyme.

The present invention also provides a method for detecting the presence of taxol or a taxol-like substance in a sample which comprises:

a) treating the sample with a monoclonal antibody capable of binding to taxol or taxol-like substances under conditions permitting the antibody to bind to taxol or the taxol-like substance and form a complex therewith;

b) removing antibodies which did not bind to taxol or the taxol-like substance; and

c) detecting the presence of the antibodies, thereby detecting the presence of taxol or the taxol-like substance in the sample.

In one embodiment the monoclonal antibody is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

The conditions permitting the binding of antibodies to taxol or taxol-like substances that may be present in a sample comprise incubation of a culture of monoclonal

antibody and the sample. Incubation can be carried out at a temperature of from about 4°C to about 37°C for a period of from one half hour to 3 hours. In the preferred embodiment the incubation is carried at room temperature for 90 minutes.

The separation of unbound from bound monoclonal antibodies can be carried out by any method known to those skilled in the art. Examples include addition of charcoal to remove unbound taxol or taxol-like substance or by precipitation of the antibody-taxol complex with an anti-mouse globulin.

The detection of bound antibodies can be accomplished by known methods including, but not limited to, precipitation of the complex comprising antibody bound to taxol or taxol-like substance or by contacting the complex with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance. Precipitation can be facilitated by known methods which include, but are not limited to addition of a ammonium sulfate solution, such as a 50% ammonium sulfate solution, or by addition of polyethylene glycol.

In one embodiment the detection step further comprises:

i) contacting the sample with a second antibody labeled with a detectable marker, wherein the second antibody is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance;

ii) removing any unbound antibody which is labeled with a detectable marker;

iii) detecting the presence of the detectable marker on

an appropriate substrate, thereby detecting the presence of taxol or a taxol-like substance in the sample.

The labeled second antibodies which are capable of binding the monoclonal antibodies of the present invention can be produced by known methods. For example, the antibodies of the present invention are mouse IgG and IgM antibodies which can be detected by any anti-mouse IgG or IgM antibodies such as goat anti-mouse antibodies.

Detectable markers useful for the above method are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In one preferred embodiment the second monoclonal antibodies are labeled with a radioactive isotope. In another preferred embodiment the second monoclonal antibodies are labeled with an enzyme.

The sample which is being examined for the presence of taxol can be derived from an animal or plant. In one preferred embodiment of this invention the sample is plant extracts. In second preferred embodiment the sample is cytosols from cells which can be derived from animals or plants.

In a further embodiment the sample is a biological fluid taken from an animal. Biological fluids which are capable of being tested for the presence of absence of compounds in an animal are well known to those skilled in the art. Examples of such fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment of this invention the

biological fluid is serum and in a second preferred embodiment of this invention the biological fluid is urine.

In one embodiment of this invention when the sample is human serum the method further comprises a dilution step where the sample is diluted approximately 1:5 with a suitable diluent. Diluents suitable for this step will be readily apparent to those skilled in the art and include, but are not limited to such diluents as PBS-Tween 20, at a concentration range of 0.1% to 0.5%, or tris-buffers. In the preferred embodiment, the diluent is 0.1% PBS-Tween 20.

The present invention further provides a method of detecting the presence of taxol or taxol-like substance in a sample which comprises:

a) contacting a predetermined amount of taxol or taxol-like substance labeled with a detectable marker with a monoclonal antibody capable of binding to taxol or taxol-like substances under conditions permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance;

b) contacting the sample with the complex of step (a) such that any taxol or taxol-like substance in the sample will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody;

c) separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance; and

d) detecting the presence of any bound, detectably labeled taxol or taxol-like substance, thereby

detecting the presence of taxol or taxol-like substance in the sample.

In one embodiment the monoclonal antibody capable of binding to taxol or taxol-like substances is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280. Detectable markers useful for labeling taxol or taxol-like substances in the above method are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In the preferred embodiment the taxol or taxol-like substance is labeled with a radioactive isotope.

The conditions permitting the binding of antibodies to the detectably labeled taxol or taxol-like substances that may be present in a sample comprise incubation of a culture of monoclonal antibody and the sample. Incubation can be carried out at temperature range of from about 4°C to about 37°C for a period of from about one half hour to 3 hours. In the preferred embodiment the incubation is carried at room temperature for 2 hours.

The bound labeled or unlabeled taxol or taxol-like substance can be separated from the unbound labeled or unlabeled taxol or taxol-like substance by any method known to those with skill in the art. Examples of such methods include addition of a charcoal solution and centrifugation, precipitation of the complex comprising monoclonal antibody and the labeled or unlabeled taxol or taxol-like substance bound thereto or by contacting the complex with an antibody capable of binding to the

monoclonal antibody which is capable of binding to the taxol or taxol-like substance.

Precipitation can facilitated by known methods which include, but are not limited to addition of an ammonium sulfate solution, such as a 50% ammonium sulfate solution, or by addition of polyethylene glycol.

The sample which is being examined for the presence of taxol can be derived from an animal or plant. In one preferred embodiment of this invention the sample is plant extracts. In second preferred embodiment the sample is cytosols from cells which can be derived from animals or plants.

In a further embodiment the sample is a biological fluid taken from an animal. Biological fluids which are capable of being tested for the presence of absence of compounds in an animal are well known to those skilled in the art. Examples of such fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment of this invention the biological fluid is serum and in a second preferred embodiment of this invention the biological fluid is urine.

In one embodiment of this invention when the sample is human serum the method further comprises a dilution step where the sample is diluted approximately 1:5 with a suitable diluent. Diluents suitable for this step will be readily apparent to those skilled in the art and include, but are not limited to such diluents as PBS-Tween 20, at a concentration range of from about 0.1% to about 0.5%, or tris-buffers. In the preferred embodiment, the diluent is 0.1% PBS-Tween 20.

The present invention also provides a method for screening for a ligand in a subject which is not being treated with taxol which comprises:

a) obtaining a sample from a subject;

b) contacting the sample with a monoclonal antibody capable of binding to taxol or taxol-like substances under conditions permitting the antibody to any taxol-like substance in the sample and form a complex therewith;

c) removing any antibodies which did not bind to the taxol-like substance; and

d) detecting the presence of antibodies in the sample, thereby detecting the presence of the ligand in the subject.

This method is useful for more closely studying the biological processes associated with the action of taxol or biologically active taxol derivatives and can be useful for identifying endogenous taxol-like substances.

In one embodiment the monoclonal antibody capable of binding to taxol or taxol-like substances is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280. The conditions permitting the binding of antibodies to taxol or taxol-like substances that may be present in a sample comprise incubation of a culture of monoclonal antibody and the sample. Incubation can be carried out at temperature range of from about 4°C to about 37°C for a period of from about one half hour to 3 hours. In the preferred embodiment the incubation is

carried at room temperature for 2 hours.

The separation of unbound from bound monoclonal antibodies can be carried out by any method known to those skilled in the art. Examples include addition of charcoal to remove unbound taxol or taxol-like substance or by precipitation of the antibody-taxol complex with an anti-mouse globulin.

The detection of bound antibodies can be accomplished by known methods including, but not limited to, precipitation of the complex comprising antibody bound to taxol or taxol-like substance or by contacting the complex with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance. Precipitation can be facilitated by known methods which include, but are not limited to addition of an ammonium sulfate solution, such as a 50% ammonium sulfate solution, or by addition of polyethylene glycol.

In one embodiment the detection step further comprises:

i) contacting the sample with a second antibody labeled with a detectable marker, wherein the second antibody is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance;

ii) removing any unbound antibody which is labeled with a detectable marker;

iii) detecting the presence of the detectable marker on an appropriate substrate, thereby detecting the presence of taxol or a taxol-like substance in the sample.

The labeled second antibodies which are capable of binding the monoclonal antibodies of the present invention can be produced by known methods. For example, the antibodies of the present invention are mouse IgG and IgM antibodies which can be detected by any anti-mouse IgG or IgM antibodies such as goat anti-mouse antibodies.

Detectable markers useful for the above method are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In one preferred embodiment the second monoclonal antibodies are labeled with a radioactive isotope. In another preferred embodiment the second monoclonal antibodies are labeled with an enzyme.

In a preferred embodiment the sample is a biological fluid taken from an animal. Biological fluids which are capable of being tested for the presence of absence of compounds in an animal are well known to those skilled in the art. Examples of such fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In a preferred embodiment of this invention the animal is human and biological fluid is serum or urine.

In another embodiment of this invention the sample is cytosols from cells derived from the subject or from cell culture.

In one embodiment of this invention when the sample is human serum the method further comprises a dilution step where the sample is diluted approximately 1:5 with a suitable diluent. Diluents suitable for this step will be readily apparent to those skilled in the art and

include, but are not limited to such diluents as PBS-Tween 20, at a concentration range of from about 0.1% to about 0.5%, or tris-buffers. In the preferred embodiment, the diluent is 0.1% PBS-Tween 20.

The present invention also provides a ligand recognized by the above-identified method. For the purposes of this invention, the "ligand" is an endogenous taxol-like substance which is capable of being bound by the monoclonal antibodies of the present invention. In one embodiment the ligand is an endogenous taxol-like substance which is bound by the monoclonal antibody produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the ligand is an endogenous taxol-like substance which is bound by the monoclonal antibody produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

In a preferred embodiment, the endogenous taxol-like substance detected by the monoclonal antibodies of this invention are polypeptides.

This invention further provides a method of quantitatively determining the amount of taxol or taxol-like substance in a sample which comprises:

a) contacting a solid support with an excess of a composition of matter comprising taxol and an appropriate carrier molecule under conditions permitting the composition of matter to attach to the surface of the solid support;

b) contacting the solid support to which the composition of matter is attached with a suitable blocking agent or buffer;

c) contacting a predetermined amount of a biological fluid sample and a predetermined amount of a monoclonal antibody capable of binding to taxol or taxol-like substances under such conditions permitting the taxol or taxol-like substance in the sample to bind to the monoclonal antibody and form a complex therewith in solution;

d) contacting the solid support to which the composition of matter is attached with the solution of step (c) under conditions permitting antibodies which do not form a complex with the taxol or taxol-like substance in the biological fluid sample to bind to the composition of matter;

e) treating the solid support so that only the composition of matter and monoclonal antibody bound thereto remain; and

f) determining the amount of antibody bound to the composition of matter, thereby determining the concentration of taxol or taxol-like substance in the biological fluid sample.

In one embodiment the monoclonal antibody capable of binding to taxol or taxol-like substances is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

Carrier molecules which are useful to bind taxol or taxol-like substances to the solid support are well known to those skilled in the art and include, but are not

limited to, proteins such as bovine serum albumin, rabbit serum albumin, keyhole limpet hemocyanin, thyroglobulin, or ovalbumin. In the preferred embodiment of this invention the carrier molecule is rabbit serum albumin.

Coating of the solid substrate with the complex comprising taxol or taxol-like substance and the carrier molecule can be carried out at 0°C to room temperature for a period of one hour to 24 hours. In the preferred embodiment the solid substrate is contacted with an excess of the complex at 4°C and stored overnight, approximately 18 hours.

Suitable blocking agents and buffers are well known to those skilled in the art and include but are not limited to, PBS-Tween 20 or PBS containing fetal calf serum or bovine serum. In the preferred embodiment the blocking is conducted with PBS containing 1% fetal calf serum. Blocking is accomplished by incubating at temperature of approximately 37°C for about 1 hour.

The conditions permitting the binding of antibodies to taxol or taxol-like substances that may be present in a sample comprise incubation of a culture of monoclonal antibody and the sample. Incubation can be carried out at room temperature for a period of one half to 3 hours. In the preferred embodiment the incubation is carried at room temperature for 90 minutes.

The separation of unbound from bound monoclonal antibodies can be carried out by any method known to those skilled in the art, including but not limited to washing several times with PBS. In the preferred embodiment, the separation is accomplished by washing three times with PBS-Tween 20.

The detection of bound antibodies can be accomplished by known methods including, but not limited to, contacting the complex with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance. For example, the antibodies of the present invention are mouse IgG and IgM antibodies which can be detected by any anti-mouse IgG or IgM antibodies such as goat anti-mouse antibodies.

The sample which is being examined for the presence of taxol can be derived from an animal or plant. In one preferred embodiment of this invention the sample is plant extracts. In second preferred embodiment the sample is cytosols from cells which can be derived from animals or plants.

In a further embodiment the sample is a biological fluid taken from an animal. Biological fluids which are capable of being tested for the presence of absence of compounds in an animal are well known to those skilled in the art. Examples of such fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment of this invention the biological fluid is serum and in a second preferred embodiment of this invention the biological fluid is urine.

This invention further provides a method of quantitatively determining the amount of taxol or taxol-like substance in a sample which comprises:

a) contacting a predetermined amount of detectably labeled taxol or taxol-like substance with a monoclonal antibody capable of binding to taxol or taxol-like substances under conditions

permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance;

b) contacting the sample with the complex of step (a) under appropriate conditions such that any taxol or taxol-like substance in the sample will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody;

c) separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance; and

d) determining the amount of bound, detectably labeled taxol or taxol-like substance, thereby determining the amount of taxol or taxol-like substance in the sample.

In one embodiment the monoclonal antibody capable of binding to taxol or taxol-like substances is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280. Detectable markers useful for labeling taxol or taxol-like substances in the above method are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In the preferred embodiment the taxol or taxol-like substance is labeled with a radioactive isotope.

The conditions permitting the binding of antibodies to

the detectably labeled taxol or taxol-like substances that may be present in a sample comprise incubation of a culture of monoclonal antibody and the sample. Incubation can be carried out at room temperature for a period of one half hour to 3. In the preferred embodiment the incubation is carried at room temperature for 2 hours.

The bound labeled or unlabeled taxol or taxol-like substance can be separated from the unbound labeled or unlabeled taxol or taxol-like substance by any method known to those with skill in the art. Examples of such methods include addition of a charcoal solution and centrifugation, precipitation of the complex comprising monoclonal antibody and the labeled or unlabeled taxol or taxol-like substance bound thereto or by contacting the complex with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance.

Precipitation can facilitated by known methods which include, but are not limited to addition of a sodium sulfate solution, such as a 50% sodium sulfate solution, or by addition of polyethylene glycol.

The sample which is being examined for the presence of taxol can be derived from an animal or plant. In one preferred embodiment of this invention the sample is plant extracts. In second preferred embodiment the sample is cytosols from cells which can be derived from animals or plants.

In a further embodiment the sample is a biological fluid taken from an animal. Biological fluids which are capable of being tested for the presence of absence of compounds in an animal are well known to those skilled in the art. Examples of such fluids include, but are not

limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment of this invention the biological fluid is serum and in a second preferred embodiment of this invention the biological fluid is urine.

In one embodiment of this invention when the sample is human serum the method above further comprises a dilution step where the sample is diluted approximately 1:5 with a suitable diluent. Diluents suitable for this step will be readily apparent to those skilled in the art and include, but are not limited to such diluents as PBS and tris-buffers.

The present invention further provides a kit for assaying for taxol or a taxol-like substance in a sample comprising in separate compartments:

a) the monoclonal antibody capable of binding to taxol or taxol-like substances;

b) a second monoclonal antibody which is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance; and

c) a standardized solution of taxol.

In one embodiment the monoclonal antibody capable of binding to taxol or taxol-like substances is produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281. In a second embodiment the monoclonal antibody is produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280. In a preferred embodiment, the kit further comprises a plate having a plurality of wells, each well

coated with a layer of a complex comprising taxol and a suitable carrier molecule.

In another embodiment, the second monoclonal antibody is labeled with a detectable marker and the kit further comprises an appropriate substrate to detect the detectable marker.

Detectable markers useful for labeling taxol or taxol-like substances in the above method are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin. Substrates useful for detecting various detectable markers are well known to those skilled in the art.

The present invention also provides a method for monitoring the treatment of a disease in a subject being treated with taxol or a taxol-like substance which comprises using the above-identified kit to determine the amount of taxol or taxol-like substance in a sample taken from the subject.

Diseases for which the above-identified kit would be useful are all those diseases for which taxol or taxol-like substances are used in the treatment. Examples of such diseases include but are not limited to breast cancer or ovarian cancer or diseases characterized by the presence of a melanoma.

The present invention further provides an anti-idiotypic monoclonal antibody of taxol or biologically active taxol derivatives. In a preferred embodiment the anti-idiotypic monoclonal antibody is an IgG_{2b}. In this application, the term "biologically active taxol derivative" is meant to encompass all substances which could be characterized as derivatives or analogues of

taxol, and which possess the biological activity of taxol, whether derived from the taxol molecule, isolated from natural products or otherwise synthetically produced.

The present invention also encompasses fragment anti-idiotypic monoclonal antibody molecules which exhibit the same activity as the above-disclosed anti-idiotypic monoclonal antibody. Examples of such fragments are well known to those of ordinary skill in the art and known as Fab, Fab' or $F(ab')_2$ antibody fragments. In a preferred embodiment the anti-idiotypic monoclonal antibody fragment is a Fab' fragment. In another preferred embodiment the anti-idiotypic monoclonal antibody fragment is a $F(ab')_2$ fragment. Methods of producing the antibody fragments are also known to those of ordinary skill in the art. By way of example, the Fab' fragment of the above-disclosed anti-idiotypic monoclonal antibody can be produced by papain digestion of the anti-idiotypic monoclonal antibody. Similarly, the $F(ab')_2$ fragment can be produced by pepsin digestion of the anti-idiotypic monoclonal antibody.

The anti-idiotypic monoclonal antibody or anti-idiotypic monoclonal antibody fragment of the present invention encompass any anti-idiotypic monoclonal antibody or fragment thereof which binds to a receptor which binds taxol or biologically active taxol derivatives. In a preferred embodiment, the receptor is a receptor on tubulin. In another preferred embodiment, the receptor is a receptor on microtubules.

In a preferred embodiment the above-defined anti-idiotypic monoclonal antibody or fragment thereof mimics taxol and biologically active taxol derivatives by promoting the assembly of tubulin into microtubules.

The present invention also provides a hybridoma which produces an anti-idiotypic monoclonal antibody of the type described above. In a preferred embodiment the hybridoma is the hybridoma designated 82H11B9F and having ATCC Accession No. HB 11548.

The present invention also provides an anti-idiotypic monoclonal antibody which is produced by the hybridoma designated 82H11B9F and having ATCC Accession No. HB 11548.

In a preferred embodiment the above defined anti-idiotypic monoclonal antibody is labeled with a detectable marker. Detectable markers useful for labeling the above defined anti-idiotypic monoclonal antibody are well known to those skilled in the art and include, but are not limited to, radioactive isotopes, enzymes, dyes, fluorescent markers or biotin.

In a preferred embodiment the anti-idiotypic monoclonal antibodies are labeled with a radioactive isotope. In a another preferred embodiment the anti-idiotypic monoclonal antibodies are labeled with an enzyme.

The present invention also provides a method of detecting taxol receptors in a sample which comprises contacting the sample with the above defined anti-idiotypic monoclonal antibody under conditions permitting the antibody to form a complex with a receptor and detecting the presence of any complex so formed.

In the practice of this method, the conditions permitting the anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature range of from about 4°C to about 37°C for a period of from about

one half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 1 hour.

In the practice of this method, detection of the presence of complex can be accomplished by known methods including, but not limited to, precipitation of the complex or by contacting the complex with an antibody capable of binding to the anti-idiotypic monoclonal antibody of the subject invention. Precipitation can be facilitated by known methods which include, but are not limited to, addition of an ammonium sulfate solution, such as a 50% ammonium sulfate solution, or by addition of polyethylene glycol.

In a preferred embodiment of this invention the sample is a biological fluid. Biological fluids which would be useful in the practice of this invention will be readily apparent to those of ordinary skill in the art. Examples of biological fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment the biological fluid is serum. In another preferred embodiment the biological fluid is tissue extracts.

The present invention further provides a qualitative immunoassay for detecting a receptor which binds taxol or biologically active taxol receptors in a sample which method comprises:

a) contacting a sample suspected of containing the taxol receptor with above defined anti-idiotypic monoclonal antibody labeled with a detectable marker under conditions permitting the formation of a complex which comprises the detectably labeled anti-idiotypic monoclonal

antibody and the receptor; and

b) detecting the presence of the complex and thereby detecting the presence of the receptor.

In the practice of this method, the conditions permitting the detectably labeled anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of detectably labeled anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature range of from about 4°C to about 37°C for a period of from about on half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 1 hour.

In a preferred embodiment of this invention the sample is a biological fluid. Biological fluids which would be useful in the practice of this invention will be readily apparent to those of ordinary skill in the art. Examples of biological fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment the biological fluid is serum. In another preferred embodiment the biological fluid is tissue extracts.

The present invention further provides a qualitative histochemical assay for detecting the presence of a taxol receptor in a biological sample which comprises:

a) contacting the biological sample with the above defined anti-idiotypic monoclonal antibody under conditions permitting the formation a complex which includes the taxol receptor and the anti-idiotypic monoclonal antibody;

b) removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex;

c) contacting the resulting sample with a detectably labeled antibody or detectably labeled antibody fragment capable of specifically binding the anti-idiotypic monoclonal antibody under conditions permitting the labeled antibody or antibody fragment to bind to the anti-idiotypic monoclonal antibody; and

d) detecting the presence of detectably labeled antibody fragment bound to the anti-idiotypic monoclonal antibody and, thereby, detecting the presence of taxol receptor in the sample.

In the practice of this method, the conditions in step (a) permitting the anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature range of from about 4°C to about 37°C for a period of from about on half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 1 hour.

The separation in step (b) of unbound anti-idiotypic monoclonal antibody from bound anti-idiotypic monoclonal antibody can be carried out by any method known to those skilled in the art. An example of such methods include precipitation of the complex with an anti-mouse globulin.

In the practice of this method, the conditions of step (c) permitting the detectably labeled anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of detectably labeled anti-idiotypic monoclonal antibody and the sample.

Incubation can be carried out at a temperature range of from about 4°C to about 37°C for a period of from about on half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 90 minutes.

In the practice of this method, detection of the presence of complex in step (d) can be accomplished by known methods including, but not limited to, precipitation of the complex or by contacting the complex with an antibody capable of binding to the anti-idiotypic monoclonal antibody of the subject invention. Precipitation can be facilitated by known methods which include, but are not limited to, addition of an ammonium sulfate solution, such as a 50% ammonium sulfate solution, or by addition of polyethylene glycol.

The practice of this method encompasses the use of monoclonal antibodies or fragment monoclonal antibody molecules which are capable of specifically binding to the anti-idiotypic monoclonal antibody described above. Methods of producing such monoclonal antibodies and antibody fragments thereof are well known to those of ordinary skill in the art. Examples of fragment monoclonal antibodies which are capable of specifically binding to the anti-idiotypic monoclonal antibody are also well known to those of ordinary skill in the art and known as Fab, Fab' or $F(ab')_2$ antibody fragments.

In a preferred embodiment of this invention the biological sample is a biological fluid. Biological fluids which would be useful in the practice of this invention will be readily apparent to those of ordinary skill in the art. Examples of biological fluids include, but are not limited to, mucus, serum, saliva, urine, blood, feces and extracts of tissue.

In one preferred embodiment the biological fluid is

serum. In another preferred embodiment the biological fluid is tissue extracts.

The present invention also provides a quantitative histochemical assay for determining the amount, present in a sample, of a receptor to which taxol or biologically active taxol derivatives bind which method comprises:

a) contacting the sample with a predetermined amount of the above defined anti-idiotypic monoclonal antibody under conditions permitting the formation of a complex which includes the taxol receptor and the anti-idiotypic monoclonal antibody;

b) removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex;

c) contacting the resulting sample with a detectably labeled antibody or a detectably labeled antibody fragment capable of specifically binding to the anti-idiotypic monoclonal antibody under conditions permitting the detectably labeled antibody or detectably labeled antibody fragment to bind to the anti-idiotypic monoclonal antibody which is part of the complex formed in (a); and

d) determining the amount of detectably labeled antibody or detectably labeled antibody fragment bound to the anti-idiotypic monoclonal antibody and thereby determining the amount of taxol receptor in the sample.

In the practice of this method, the conditions in step (a) permitting the anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature

range of from about 4°C to about 37°C for a period of from about on half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 1 hour.

The separation in step (b) of unbound anti-idiotypic monoclonal antibody from bound anti-idiotypic monoclonal antibody can be carried out by any method known to those skilled in the art. An example of such methods include precipitation of the complex with an anti-mouse globulin.

In the practice of this method, the conditions of step (c) permitting the detectably labeled anti-idiotypic monoclonal antibody to form a complex with the receptor comprise incubation of a culture of detectably labeled anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature range of from about 4°C to about 37°C for a period of from about on half hour to 3 hours. In the preferred embodiment the incubation is carried out at 37°C for 1 hour.

The practice of this method encompasses the use of monoclonal antibodies or fragment monoclonal antibody molecules which are capable of specifically binding to the anti-idiotypic monoclonal antibody described above. Methods of producing such monoclonal antibodies and antibody fragments thereof are well known to those of ordinary skill in the art. Examples of fragment monoclonal antibodies which are capable of specifically binding to the anti-idiotypic monoclonal antibody are also well known to those of ordinary skill in the art and known as Fab, Fab' or $F(ab')_2$ antibody fragments.

The present invention also provides a method for determining the amount of taxol or biologically active taxol derivative in a sample which comprises:

a) immobilizing the above defined anti-idiotypic monoclonal antibody on an appropriate substrate;

b) contacting the sample containing taxol or biologically active taxol derivative with the immobilized anti-idiotypic monoclonal antibody of step (a) under conditions permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized anti-idiotypic monoclonal antibody;

c) contacting the complex formed in step (b) with a predetermined amount of a labeled antibody or labeled antibody fragment which specifically binds to taxol or biologically active taxol derivative under conditions permitting the labeled antibody or labeled antibody fragment to displace taxol or biologically active taxol derivative bound to the immobilized anti-idiotypic monoclonal antibody; and

d) determining the amount of labeled antibody or labeled antibody fragment bound to the immobilized monoclonal anti-idiotypic monoclonal antibody thereby determining the amount of taxol or biologically active taxol in the sample.

Examples of suitable solid supports useful for immobilizing the anti-idiotypic monoclonal antibody are well known to those of ordinary skill in the art. Examples of supports include, but are not limited to, such materials as aminohexol sepharose and commercial products such as Affi-gel 10® (available from Bio-Rad) and Reacti-gel® (available from Pierce).

In the practice of this invention the conditions in step (b) permitting any taxol or biologically active taxol derivative in the sample to form a complex with the

immobilized anti-idiotypic monoclonal antibody comprise incubation of a culture of the bound anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature of about 0°C for a period of 12 to 24 hours. In the preferred embodiment the incubation is carried out at a temperature of 0°C for 18 hours.

In the practice of this invention the conditions in step (c) permitting the labeled antibody or labeled antibody fragment to displace taxol or biologically active taxol derivative bound to the immobilized anti-idiotypic monoclonal antibody comprise incubation of a culture of the complex formed in step (b) with the predetermined amount of labeled antibody or labeled antibody fragment. Incubation can be carried out at a temperature of about 0°C for a period of 12 to 24 hours. In the preferred embodiment the incubation is carried out at a temperature of 0°C for 18 hours.

In a preferred embodiment of this method the labeled antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280 labeled with a detectable marker.

In another preferred embodiment of this method the labeled antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281 labeled with a detectable marker.

The practice of this method encompasses the use of detectably labeled fragment monoclonal antibody molecules which are capable of specifically binding taxol or biologically active taxol derivatives. Methods of producing such antibody fragments are well known to those

of ordinary skill in the art including such methods as enzyme digestion of the monoclonal antibody. In a preferred embodiment the detectably labeled monoclonal antibody fragment molecule is a fragment of the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280 labeled with a detectable marker. In another preferred embodiment the detectably labeled monoclonal antibody fragment molecule is a fragment of the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281 labeled with a detectable marker.

The present invention further provides a method for determining the amount of taxol or biologically active taxol derivative in a sample which comprises:

a) immobilizing an antibody or antibody fragment which specifically binds to taxol or biologically active taxol derivatives on an appropriate substrate;

b) contacting the sample containing taxol or biologically active taxol derivative with the immobilized antibody or antibody fragment of step (a) under conditions permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized antibody or antibody fragment;

c) contacting the complex formed in step (b) with a predetermined amount of the above defined anti-idiotypic monoclonal antibody labeled with a detectable marker under conditions permitting the labeled anti-idiotypic monoclonal antibody to displace any taxol or biologically active taxol derivative bound to the immobilized antibody or antibody fragment; and

d) determining the amount of labeled anti-idiotypic monoclonal antibody bound to the immobilized antibody or antibody fragment thereby determining the amount of taxol or biologically active taxol derivative in the sample.

Examples of suitable solid supports useful for immobilizing the antibody or antibody fragment of step (a) are well known to those of ordinary skill in the art. Examples of supports include, but are not limited to, such materials as aminohexol sepharose and commercial products such as Affi-gel 10® (available from Bio-Rad) and Reacti-gel® (available from Pierce).

In the practice of this invention the conditions in step (b) permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized antibody or antibody fragment comprise incubation of a culture of the bound anti-idiotypic monoclonal antibody and the sample. Incubation can be carried out at a temperature range of from about 0°C to about 37°C for a period of from about 2 hours to about 24 hours. Those of ordinary skill in the art will recognize that as the temperature of the incubation is raised, the time of incubation decreases. Accordingly, in a preferred embodiment the incubation is carried out at a temperature of 37 °C for a period of about 2 hours.

In the practice of this invention the conditions in step (c) permitting the labeled anti-idiotypic monoclonal antibody to displace any taxol or biologically active taxol derivative bound to the immobilized antibody or antibody fragment comprise incubation of a culture of the complex formed in step (b) with the predetermined amount of the above defined anti-idiotypic monoclonal antibody labeled with a detectable marker. Incubation can be carried out at a temperature range of from about 0°C to

about 37°C for a period of from about 2 hours to about 24 hours. Those of ordinary skill in the art will recognize that as the temperature of the incubation is raised, the time of incubation decreases. Accordingly, in a preferred embodiment the incubation is carried out at a temperature of 37 °C for a period of about 2 hours.

In a preferred embodiment of this method the antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281.

In another preferred embodiment of this method the antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280.

The practice of this method encompasses the use of fragment monoclonal antibody molecules which are capable of specifically binding taxol or biologically active taxol derivatives. Methods of producing such antibody fragments are well known to those of ordinary skill in the art including such methods as enzyme digestion of the monoclonal antibody. In a preferred embodiment the monoclonal antibody fragment molecule is a fragment of the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280. In another preferred embodiment the monoclonal antibody fragment molecule is a fragment of the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281.

The invention is further illustrated in the Experimental Details section which follows. The Experimental Details section and Examples contained therein are set forth to aid in an understanding of the invention. This section

is not intended to, and should not be interpreted to, limit in any way the invention set forth in the claims which follow thereafter.

Experimental Details

EXAMPLE 1

Production of monoclonal antibodies.

I. Materials and Methods

A. Reagents

Taxol (NSC-125973), cephalomannine (NSC-318735), baccatin III (NSC-330753) and [3H]taxol (23 Ci/mmol) (NSC-125973) were obtained from the National Cancer Institute. The following taxol derivatives were a generous gift from Dr. D.G.I. Kingston (Virginia Polytechnic Institute and State University, Blackburg, VA): 2'-(triethylsilyl)taxol, 7-epitaxol, 2-debenzoylisotaxol, 2-[N-benzyloxycarbamyl (Cbz)]-β-alanyl)-7-oxo-5,6-dehydro-5-0-secotaxol, 20-acetoxy-4-deacetyl-5-epi-20,0-secotaxol, 10-deacetylbaccatin III and 7-(triethylsilyl) baccatin III. We thank Dr. P. Potier of the Institut de Chimie des Substances Naturelles (CNRS), Gif-Sur-Yvette, France and Dr. J.-L. Fabre of Rhone-Poulenc Rorer (France) for the sample of taxotere.

Bovine serum albumin (BSA), rabbit serum albumin (RSA), charcoal, polyvinylpyrrolidone (PVP) and succinic anhydride were purchased from Sigma Chemical Corp. (St. Louis, MO). Isobutylchloroformate and n-tributylamine were from Eastman Kodak Corp. (Rochester, NY). Dextran T70 was purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). Fetal calf serum (FCS) was from Hyclone (Logan, UT). Peroxidase-conjugated goat antimouse IgG+IgM was purchased from TAGO (Burlingame, CA). The isotyping kit was from Zymed (San Francisco, CA).

B. Synthesis of 2'-hemisuccinyltaxol

The method of Deutsch et al. (11) was used with some modifications. Taxol (20 mg) and succinic anhydride (36 mg) were dried for 4 hours at room temperature under vacuum over P_2O_5 and dissolved in 480 ul of dry pyridine. After standing at room temperature overnight, the pyridine was removed under vacuum and the residue washed one with 2 ml of distilled water. Acetone (1 ml) was added, and distilled water was added dropwise to the acetone solution until a few crystals (2-hemisuccinyltaxol) appeared. The mixture was kept at 4°C for 3 hours and the crystals were recovered by filtration and dried under vacuum. The product was obtained in 70% yield.

C. Synthesis of 2'-hemisuccinyltaxol-protein conjugates

A modification of the procedures developed by Jaziri et al. (12) was used. 2'-hemisuccinyltaxol (10 mg) was dissolved in 1 ml DMSO and 300 ul acetonitrile, and 50 ul (35 mg, 0.19 mmoles) of n-tributylamine was added. The mixture was cooled to 4°C in a ice bath, and 25 ul (25 mg, 0.18 mmoles) of isobutylchloroformate was added to the mixture which was kept in the ice bath for another 30 min.

The solution was added dropwise into a BSA or RSA solution (25 mg, [3.73 x 10^{-4} mmoles] in 3 ml distilled H_2O, pH=9.5, at 4 °C). The pH was adjusted immediately to 7.5 with 1 N HCI and the mixture kept at 4°C overnight and dialyzed against PBS at 4°C overnight.

D. Rabbit antibodies

A female New Zealand White Rabbit was immunized intradermally along the back, with a 1:1 (v/v) mixture of

1 mg of 2'-hemisuccinyltaxol-BSA conjugate (taxol-BSA) in PBS and complete Freund's adjuvant (CFA). The rabbits were boosted with 0.5 mg of taxol-BSA incomplete Freud's adjuvant (IFA) at 3-4 week intervals and bled weekly following each boost.

E. Monoclonal antibodies (MAbs)

BALB/c mice (Charles River) were immunized i.p. with 0.5 mg taxol-BSA emulsified in CFA. Mice were boosted twice at two or three week intervals with 0.25 mg of taxol-BSA emulsified in IFA. Five days before the fusion, the mice were injected i.p. with 0.25 mg of taxol-BSA in PBS. Spleen cells were fused with nonsecreting myeloma cells P3 x 63-Ag8.653 (13), according to the method of Sharon et al. (14). Three weeks later, the hybridoma supernatant was assayed for the presence of anti-taxol antibodies by ELISA (see below). The positive clones were confirmed for taxol binding by a competitive ELISA (see below). Clones positive by competitive ELISA were subcloned twice by limiting dilution. Ascites were obtained by injecting 10^6 to 10^7 cells i.p. into BALB/c mice that had been primed with IFA i.p. 5 days before.

F. ELISA for anti-taxol MAb screening

Polystyrene microplates (Corning 25855) were coated with 100 ul of taxol-RSA (250ng/ml) in 0.1 M sodium bicarbonate, PH=9.3, overnight at 4°C. The plates were washed with PBS containing 0.1% Tween 20 (PBS-T-20) three times, and 100 ul of culture supernatants were incubated in the wells for 2 hours at 37°C. The plates were washed three times with PBS-T-20 and 100 ul of a 1/3000 dilution of horseradish peroxidase-labeled goat anti-mouse IgG+IgM in PBS-Tween 20 was added to each well and incubated at 37°C for 1 hour. After washing the plates three times with PBS-Tween 20, 100 ul of substrate (7 mg o-

phenylenediamine dihydrochloride in 10 ml of 0.1 M citrate-phosphate buffer, pH=5, containing 5 ul of 30% H_2O_2, was added to each well. The reaction was stopped after 10 min by the addition of 40 ul of 8N H_2SO_4, and the absorbance of each well measured at 490 nm on a Dynatech Microplate reader.

G. Competitive ELISA

Polystyrene microplates were coated with 100ul of taxol-RSA (250ng/ml) in 0.1 M sodium bicarbonate, pH=9.3, overnight at 4°C. The wells were washed with PBS-T-20 three times and blocked with 200 ul of PBS, containing 1% fetal calf serum, for 1 hour at 37°C. Culture supernatant (100ul) was added to the coated plate either in the presence or in the absence of 50 uM taxol in PBS-T-20 (from a 10 mM taxol stock sodium in dimethyl sulfoxide), followed by incubation at room temperature for 90 min. After washing four times with PBS-T-20, bound antibodies were detected with 100 ul of 1/3000 dilution of peroxidase-labeled goat anti-mouse IgG+IgM in PBS-T-20 for 1 hour at 37°C. Color was developed and absorbance was measured as described above.

For those dose dependent inhibition of binding of anti-taxol to taxol-RSA, 100 ul of diluted MAb IgM (29B73C) or MAb IgG (69E4A8E) ascites was added to the coated well with serial dilutions of taxol or its derivatives, from 0.1 mM to 0.24 nM (all derivatives were from a 10^{-2}M stock solution in DMSO), in PBS-T-20+2.5% FCS+3.5% polyvinyl pyrrolidone (PVP)+1% DMS).

II. Determination of taxol in human serum

A. ELISA

First a standard curve was determined by adding a mixture of 50 ul of 1/8000 dilution of 69E4A8E ascites in PBS-T-20 and 50 ul of serial 5-fold dilutions of taxol (from 0.1 mM to 0.24 nM) in PBS-T-20 into the taxol-BSA-coated wells.

To measure taxol levels in human serum, different amounts of taxol in DMSO were added to human serum; the final concentration of DMSO was, in all cases, 0.5%. A mixture of 50 ul of 1/8000 dilution of 69E4A8E ascites and 50 ul of a 1/5 dilution of serum in PBS-T-20 was added to taxol-RSA-coated plates, followed by incubation at room temperature for 90 min. Bound antibodies were detected as described above.

B. Radioimmunoassay

For a standard curve of anti-taxol antibody binding to [^{3}H]taxol, 100 ul of diluted 29B7B3C or 69E4A8E ascites or rabbit antiserum in RIA buffer (PBS+0.1% T-20+0.1% gelatin+0.1% NaN_3) was incubated for 2 hours at room temperature with 100 ul of [^{3}H]taxol (Ca. 10,000 cpm) in RIA buffer, in the presence of 100 ul of serially diluted taxol solutions in RIA buffer. Bound ligand was separated from free by the addition of 100 ul of a 2.5% dextran-coated charcoal solution in RIA buffer, incubation for 3 min at 4°C and centrifugation in an Eppendorf centrifuge for 2 min. The supernatant, containing bound [^{3}H]taxol, was counted for radioactivity. For characterizing the antiserum, taxol derivatives were incubated at room temperature for 2 hours with the rabbit antiserum and [^{3}H]taxol. Bound [^{3}H]taxol was determined as described above.

To measure taxol levels in human serum by RIA, 100 ul of 1/150 dilution of rabbit anti-taxol antiserum or 1/150 dilution of 69E4A8E ascites were added to 100 ul of [^{3}H]taxol in RIA buffer and 100 ul of undiluted to 1/100 dilution of human serum samples originally containing concentrations of taxol from 0.005 uM to 5 uM. For the higher concentrations, the sera were diluted with RIA buffer to bring the concentrations within the working range of the RIA (0.03 nM to 10 nM). After incubating for 2 hours at room temperature, bound [^{3}H]taxol was determined as described above.

III. Results

A. Characterization of antibodies

Antibodies generated in rabbits using a taxol-BSA conjugate wee assayed for specificity by RIA (Figure 1). The antibodies bound taxol and cephalomannine with almost equal affinity. Two inactive derivatives, baccatin III and 20, O-secotaxol, were bound with affinities about 3 orders of magnitude lower than taxol. Taxotere, a biologically active compound (15, 16), was bound with 100-fold lower affinity than taxol.

Two mouse monoclonal anti-taxol antibodies were isolated, 29B7B3C (IgM) and 69E4A8E (IgG_1). Taxol inhibited the binding of both antibodies to taxol-R3A, as shown by ELISA, with an IC_{50} for taxol of about 0.1 uM. The specificities of the antibodies were determined by a competitive ELISA with taxol and 10 related derivatives. (Figures 2 and 3). Because many of the derivatives were not soluble in 5% DMSO at their high concentrations, PVP (3.5%) was introduced into the solution. Yonish-Rouach et al. (17) found that cyclosporin A, which is not soluble in water, could be solubilized in an aqueous solution containing 3.5% PVP (PVP (PH=7.4) without

affecting immunological assays of cyclosporin A. We found that taxol and its derivatives were also more soluble in the presence of 3.5% PVP without any deleterious effect on the immunoassays (data not shown).

The IC_{50} of each derivative, as determined by the ELISA inhibition assays (Figures 2 and 3), are shown in Table 1. Both monoclonal antibodies had higher binding affinities for biologically active derivatives (taxol, cephalomannine and 7-epitaxol) than for inactive derivatives (baccatin III derivatives and derivatives with an open oxetane ring). Specificity was consistent with the results of tubulin disassembly assays and cytotoxicity studies using the same taxol derivatives (10). An exception was the biologically active derivative taxotere which was recognized poorly by 69E4A8E. This had also been the case with the rabbit antiserum (see above).

B. Measurement of taxol levels in human serum by ELISA and RIA

For these experiments, known amounts of taxol were dissolved in human serum.

Taxol levels in human serum were measured by RIA using the rabbit antiserum. The results are in Table 2. The lowest concentration of taxol detected was 5 nM. However, the lower limit of measurement, as determined from the standard inhibition curve was 0.1 nM (0.085 ng/ml). The MAbs could also be used to measure taxol levels by RIA, but the lower limit of measurement was 50 nM (data not shown).

Taxol levels in human serum could be also measured by ELISA using 69E4A8E (Table 3). In preliminary experiments, we found that undiluted human serum

partially blocked the binding of antibody to taxol-RSA as measured by ELISA. Interference of binding was minimized by a 1:5 dilution of the serum with PBS. The lower limit of measurements was about 50 nM or 42 ng/ml.

IV. Discussion

All of the antibodies are sensitive to the presence of the side chain ester at C-13 and an intact oxetane ring. In other words, biologically active compounds are bound well and inactive derivatives are bound poorly. An exception is the inability of the rabbit serum and 69E4A8E to recognize taxotere, a semi-synthetic biologically active taxol analogue. The differences between taxol and taxotere lacks an C-19 acetyl group and has a t-butyloxycarbamido group rather than a benzamido group at the C-3' position. It is likely to be the latter that is the significant difference because the C-10 acetyl group is not necessary for activity. Moreover, cephalomannine, which is acetylated at C-10, is more poorly recognized by 69E4A8E. Apparently, the phenyl ring of taxol is an important determinant group for binding to the rabbit antibodies and to 69E4A8E.

On the other hand, 29B7B3C binds taxotere as well as it does taxol and it does not bind inactive derivatives well. We suggest, therefore, that it should be possible to use this antibody to screen for taxol or taxol-like compounds in extracts of natural products. We have begun to investigate this possibility. Moreover, its interaction with active taxol-related compounds closely correlates with their effects on microtubulin disassembly, making 29B7B3C an excellent candidate for eliciting anti-idiotypic antibodies that mimic taxol (22).

Our antibodies can measure taxol levels in human serum to

which known quantities of taxol were added. In clinical trials, HPLC has been used to measure taxol levels in serum, urine and other biofluids, the lower limit of detection being 50 nM (9, 23). However HPLC techniques are not as suitable as immunoassays for routine analysis of large numbers of samples of biological fluids. The only immunoassay reported thus far is that of Jaziri et al. (12). Their rabbit antiserum could detect as little as 23.5 nM or 20 ng/ml in plant extracts by ELISA. They did not examine human serum. Our monoclonal antibodies could measure taxol in concentration range of about 10nM to 1um in PBS. However, the presence of human serum interfered with the binding of the antibodies in ELISA assays, requiring a dilution step that decreased the sensitivity of the procedure to a lower limit of 50nM. The interfering factor in human serum did not seem to be an endogenous taxol-mimicking substance, because, upon dilution, its inhibition curve was not similar to that of taxol. Moreover, serum did not interfere with the RIAs. We will be investigating this further.

Rowinsky and Donehower (9) reviewed pharmacokinetic studies of taxol. In the doses recommended in phase II trials, i.e. 200 to 250 mg/m^2 infusion over 24 hours, the peak taxol level in plasma were above 0.6 uM, well within the range detectable and measured by our antibodies.

Table 1. Relative IC_{50} of taxol derivatives in competitive ELISA, tubulin dissassembly and cytotoxicity assays

	Competitive ELISA		Tubulin disassembly[a]	Cytotoxicity[a] (KBcells)
	29B7B3C	69E4A8E		
Taxol	1	1	1	1
Cephalomannine	0.4	44.2	1.5	3.2
7-Epitaxol	1.7	3.1	3	3
Taxotere	1.2	1000	0.5	0.4[b]
2'-(Triethylsilyl)taxol	33.2	153.8	----	30,000[c]
2'(N-cbz-B-alanyl) 7-oxo5,6-dehydro-5-0-secotaxol	>500	>1000	----	-----

[a] All data are from reference 10.

[b] This value was from experiments using J774.2 cells; no data for KB cells is available.

[c] This value was from experiments using 2'-(t-butyldimethylsilyl)taxol, which is similar in structure to 2'-(triethylsilyltaxol).

Table 1 Continued.

	Competitive ELISA		Tubulin disassembly[a]	Cytotoxicity[a] (KBcells)
	29B7B3C	69E4A8E		
20-Acetoxy-4-deacetyl-5-epi-20,O-secotaxol	473.7	>1000	>21	>100,000
2'-Debenzoylisotaxol	>500	>1000	----	----
Baccatin III	63.2	>1000	52	1,700
10-deacetylbaccatin III	63.2	>1000	46	400
7-(Triethylsilyl)-baccatin III	>500	>1000	384[d]	----

[a] All data are from reference 10.

[d] This value was from experiments using 7-acetylbaccatin III

Table 2. Measurement of taxol levels in human serum by RIA using rabbit antitaxol antiserum.

Actual taxol concentrations	Taxol concentrations found[a]
5 uM	5.87 ± 1.00 uM
500 nM	476 ± 4 nM
50 nM	32.7 ± 0.3 nM
10 n M	11.3 ± 0.5 nM
5 nM	5.67 ± 0.64 nM

[a] All samples were done in duplicate.

Table 3. Measurement of taxol levels in human serum by ELISA using 69E4A8E.

Actual taxol concentrations	Taxol concentrations found[a]
5 uM	3.61 ± 0.35 uM
500 nM	600 ± 144 nM
50 nM	62.1 ± 9.8 nM

[a] Data were averaged from three duplicate experiments for each concentration.

EXAMPLE 2

Production of anti-idiotypic monoclonal antibody.

I. Materials and Methods

A. Affinity purification of rabbit polyclonal anti-taxol antibodies

Rabbit anti-taxol antiserum (R585) was prepared and characterized as described before (24). 10 milligrams each of taxol-RSA (rabbit serum albumin) (24) and BSA (bovine serum albumin) in 1 ml of 0.1 M 2-(N-morpholino)-ethanesulfonic acid (MES), pH = 4.8, were added to 2 ml of Affigel-10, respectively, and rocked overnight at 4°C. The gel was washed with several column volumes of PBS (pH 7.3) and with 0.2 M glycine-HCl buffer, pH 2.5, and then equilibrated with PBS. 6 ml of rabbit polyclonal anti-serum was first recirculated through a taxol-RSA-Affigel 10 column overnight at 4°C at a rate of 0.2 ml/min. The column was then extensively washed with PBS at a rate of 1.0 ml/min to remove nonspecific bound proteins, and the antibodies were eluted with 0.2 M glycine-HCl, pH=2.5, at a rate of 0.5 ml/min. The eluted antibodies were immediately neutralized with 2 M Tris base and dialyzed against PBS at 4°C overnight. On the next day, the eluted antibodies were recycled through a BSA-Affigel 10 column at 4°C overnight to remove BSA-binding antibodies. The effluent which contained anti-taxol antibodies in PBS was collected and concentrated by a Centriprep-10 concentrator (Amicon) for following use.

B. Preparation of Fab fragments of rabbit anti-taxol antibodies

Affinity-purified antibodies in PBS (0.01 M NaHP04-0.15 M NaCL were digested by 1/100 (w/w) of mercuripapain in

the presence of 2 mM EDTA and 2 mM dithiotreitol overnight at 37°C. The solution was recirculated through a Protein A-Sepharose-4B column at 4°C overnight. The undigested IgG and Fc fragments remained bound to the column and Fab fragments were eluted. The purity of the Fab fragments was confirmed by SDS-polyacrylamide gel electrophoresis.

C. Hybridomas

Balb/c mice were immunized and boosted several times with taxol-BSA as described before **(24)**. Cell fusion was done and supernatants were screened by ELISA. The 96-well microplates were coated with 100 µl of 0.5 µg/ml of rabbit Fab fragments in 0.1 M $NaHCO_3$, pH = 9.3, at 4°C overnight. The wells were washed with PBS + 0.1% Tween 20 (PBS-T-20) three times and blocked with 200 µl of 1% fetal calf serum (F.C.S.) in PBS-T-20 for 1 hour at 37°C. After washing three times with PBS-T-20, 100 µl of culture supernatant were added to the wells and incubated at 37°C for 2 hours. The plates were washed three times with PBS-T-20, and 100 µl of a 1/3000 dilution of horseradish peroxidase-labeled goat anti-mouse IgG + IgM in PBS-T-20 were added to each well and incubated at 37°C for 1 hour. After the plates were washed three times with PBS-T-20, 100 µl of substrate (7 mg o-phenylenediamine dihydrochloride in 10 ml of 0.1 M citrate-phosphate buffer, pH 5, containing 5 µl of 30% H_2O_2) were added to each well. The reaction was stopped after 10 min by the addition of 40 µl of 8N H_2SO_4, and the absorbance of each well was measured at 490 nm on a Dynatech Microplate reader. Positive wells were confirmed by competitive ELISA.

D. Competitive ELISA

The 96-well microplates were coated with 100 µl of 0.5

μg/ml of rabbit Fab fragments in 0.1 M $NaHCO_3$, pH = 9.3, at 4°C overnight. After washing with PBS-T-20 three times and blocking with 1% F.C.S. at 37°C for 1 hour, 100 μl of culture supernatant was added to the coated wells with or without 0.1 mM taxol in PBS-T-20 (from a 10 mM taxol stock solution in dimethyl sulfoxide), followed by incubation at 37°C for 2 hours. After washing four times with PBS-T-20, bound antibodies were detected with 100 μl of 1/3000 dilution of peroxidase-labeled goat anti-mouse IgG+IgM in PBS-T-20 for 2 hour at 37°C. Color was developed and absorbance was measured as described above. Cells in the wells which showed inhibition by taxol were subcloned twice.

In characterizing the monoclonal anti-idiotypic antibody, the plates were coated with rabbit Fab fragments as described above. After blocking with F.C.S., 100 μl of culture supernatants were added to the coated wells with serial dilutions of taxol and taxol analogues, followed by incubation at 37°C for 2 hours. Bound antibodies were detected as described above. Antibodies whose binding to the Fab fragments was inhibited by taxol were selected for study.

E. Affinity-purified mouse monoclonal anti-idiotypic antibodies to taxol

Monoclonal hybridoma cells were cultured in medium with 5% fetal calf serum. The supernatant was collected every other day. For purifying antibodies, 150 ml of culture supernatant (pH = 8.0) was recycled through a 2 ml protein A-sepharose 4B affinity column (Sigma) at 4°C, 0.2 ml/min, overnight. After washing the column with PBS (pH = 7.3), the bound IgG antibodies were eluted with 0.1 M citric acid (pH = 3.0) and the eluate was neutralized by 2 M tris base immediately. The eluate was dialyzed against PBS at 4°C overnight and antibodies were

concentrated by Centriprep-10 concentrators.

F. Preparation of microtubules

Microtubules were purified from fresh calf brain by modified procedures from Shelanski et al. **(25)** Calf brain was minced and homogenized in 4°C MES buffer (0.1 M MES, 0.5 mM $MgCl_2$ and 1 mM EGTA, pH = 6.6). After centrifugation at 15,000 x g at 4°C for 20 min, the supernatant was collected and centrifuged again at 4°C, 200,000 x g, for 40 min. Tubulin in supernatant was polymerized at 37°C for 30 min, in the presence of 1 mM GTP and 4 M glycerol. Polymerized microtubules were spun down at 200,000 x g at 30°C for 30 min, and pellets were either stored at -70°C for further use or purified again. In the second purification, the once purified pellets were resuspended and homogenized in cold (4°C) MES buffer for 30 min, followed by centrifugation at 200,00 x g, 4°C, for 30 min. The supernatant was stored at -70°C with 8 M glycerol and 1 mM GTP. Before each experiment, the stored solution was mixed with equal volume of MES buffer containing 2 mM GTP and incubated at 37°C for 30 min. After centrifugation at 30°C, 200,000 x g, the pellets were used for the experiments.

G. Radioimmunoassay with microtubules

The microtubule pellets after one purification were resuspended in MES buffer + 3 mM GPT of 4°C for 30 min to make microtubules depolymerize to tubulin, followed by centrifugation at 100,00 x g, 4°C, for 20 min. The supernatant was used for radioimmunoassay and the protein concentration in the supernatant was measured by Bradford method **(36)** using an assay solution from Bio-rad.

Inhibition of [^{3}H]taxol binding to microtubules was performed by a method described by Parness et al. **(19)**

with some modification. 400 μl of tubulin (1.5 mg/ml) in MES buffer + 3 Mm GTP were put in each ultracentrifuge tube (5 ml, Nalgene) and incubated at 37°C for 40 min. 350 μl serial dilutions of antibodies or taxol analogues in PBS + 0.1% NaN_3 were added to each corresponding tube and incubated at 37°C for 30 min. 50 μl of [^{3}H]taxol in MES buffer + 3 Mm GTP were added to each tube and incubated at 37°C for another 45 min. All tubes were centrifuged at 100,000 x g, 30°C, for 20 min. The supernatant of each tube was discarded. The pellets were washed once with warm (37°C) MES buffer + 10% sucrose and dissolved by 400 μl of 0.1 N NaOH with vigorous vortex. An aliquot of 165 μl was taken from each tube for counting radioactivity.

A turbidometric assay for microtubule formation described by Parness et al. **(19)** was used with modification. Tubulin (1mg/ml) after two purified in MES buffer without GTP was prepared. 480 μl of antibodies or taxol in PBS + 0.1% NaN_3 were put in each corresponding cuvette. The tubulin solution and the cuvettes with reagents were warmed up at 37°C for 5 min. After heating, 620 μl of tubulin solution (1 mg/ml) was added to each cuvette and mixed up immediately. The mixtures were kept in 37°C water bath and the turbidity change was measured every 5 min with a spectrophotometer at 352 nm.

H. Electron microscopy

One drop of the samples from the microtubule assembly assay was put on a grid and stayed for 15 seconds. After removing the excess solution by a piece of filter paper, one drop of 1% uranyl acetate was put on the grid to stain. The excess uranyl acetate was immediately removed by filter paper. The grid was dried by air at room temperature for several hours and checked by an electron microscope.

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II. Results

A. Characterization of mouse monoclonal anti-idiotypic antibodies

A mouse monoclonal anti-idiotypic antibody of taxol, 82H11B9F, was found. This antibody, an IgG_{2b}, could bind to the Fab fragments of affinity-purified rabbit polyclonal anti-taxol antibodies and the binding could be inhibited by free taxol in competitive ELISA, with an IC_{50} around 3μM (Figure 4). To confirm that this antibody mimicked the active part of taxol molecules, a competitive ELISA with active and inactive taxol analogues was done. The binding of 82H11B9F to the coated rabbit Fab fragments could be inhibited by an active analogue taxotere, but not inhibited by the inactive analogue, baccatin III. These results showed that 82H11B9F mimicked the microtubule-binding part of taxol and active analogues. The IC_{50} for taxotere was about 30 mM which was less than that of taxol. In previous reports, however, taxotere showed better potency than taxol in cytotoxicity and microtubules disassembly inhibition assay **(15,16)**. Although not wanting to be limited to any particular theory, we believe the reason for this difference is that the rabbit anti-taxol antibodies are more specific for taxol **(24)** and, therefore, free taxol will inhibit the binding of 82H11B9F to the Fab fragments.

B. Radioimmunoassay with microtubules

To determine whether 82H11B9F had the "internal image" to the active part of taxol, two criteria were set up. One was that the binding of taxol to microtubules should be inhibited by 82H11B9F, the other was that 82H11B9F should have the ability to polymerize tubulin to microtubules as taxol. Radioimmunoassay with microtubules was used to

confirm the first definition.

Radioimmunoassay was performed in previous experiments using taxol analogues and antitumor agents in MES buffer (pH = 6.6) **(4, 19, 26)**. We also attempted the experiments with 82H11B9F in MES buffer with the same pH value, but the activity of 82H11B9F was found to disappear if it was stored in MES buffer (pH 6.6), PIPES buffer (pH 6.8) or PBS (pH 6.6). Therefore, the PBS buffer with the pH value of 7.3 was chosen to run the experiments with 82H11B9F. Because the polymerization of tubulin to microtubules was unsatisfactory in PBS buffer, MES buffer and a greater amount of GTP (3 mM) was used in the first step of the experiments to be sure that enough microtubules could be polymerized. After adding 350 ml of inhibitors in PBS to 400 ml of microtubules in MES buffer, some polymerized microtubules would depolymerize to tubulin based on our observation that the available binding sites for [^{3}H] taxol decreased. Most microtubules, however, were still stable in the mixture. The final pH value was detected to be 6.75.

We also observed that [^{3}H] taxol readily adheres to the wall of the ultracentrifuge tubes and the radioactivity in solution decreased dramatically after incubation at 37°C for 30 min. Therefore, 0.3% of Tween-20 was added in the 50 μl of MES buffer with [^{3}H] taxol and the final concentration of Tween-20 in the final volume of 800 μl was 0.02%. At this concentration, Tween-20 could prevent the adherence of [^{3}H] taxol to the tube and not affect the standard inhibition curve of cold taxol.

Taxol and two taxol analogues, taxotere and baccatin III, were used to check whether the mixture of PBS and MES buffer could provide a good environment for radioimmunoassay. Taxol and the active analogue, taxotere, showed inhibition for the binding of [^{3}H]taxol

to microtubules, but the inactive analogue, baccatin III, did not (Figure 5A). The IC_{50} of taxol were around 4 μM, which was compatible with the data reported for the similar methods **(19)**.

The IC_{50} of taxotere was about 2 μM, which was better than that of taxol. Ringel and Horwitz **(15)** reported that taxotere was more potent than taxol in cytotoxicity and tubulin polymerization. It seemed that taxotere had higher binding affinity to microtubules than taxol, although no radioimmunoassay using taxotere was reported before. The bad water solubility of taxol also seemed to affect its inhibition to [^{3}H]taxol binding. In previous reports, with a similar method, the peak inhibition of taxol to [^{3}H] taxol was only 75-80% with taxol concentration of more than 10 μM **(19,27)**. In addition, the adherence of cold taxol to the ultracentrifuge tubes also decreased the inhibition. We found that the inhibition of taxol at 13 μM could be 80% instead of 71%, if 0.03% tween-20 was added with cold taxol.

Affinity-purified 82H11B9F showed inhibition for the binding of [^{3}H]taxol to microtubules with an IC_{50} around 1 μM, which was better than the IC_{50} of taxol and taxotere described above (Figure 5B). In contrast, the unrelated control antibody 26F, which was also IgG_{2b} and against thymine glycol **(28)**, only showed slight inhibition at the highest concentration.

We reported two mouse monoclonal anti-taxol antibodies **(24)**. These antibodies were able to bind taxol and also prevent the binding of [^{3}H]taxol to microtubules in the same experiments. Therefore, 82H11B9F was checked to see whether it had the same character as the anti-taxol antibodies and showed the inhibition. A radioimmunoassay without microtubules was performed. 82H11B9F was incubated with [^{3}H]taxol at 37°C for 1 hour at the same

buffer mixture and the bound [^{3}H]taxol was separated from the free by charcoal. No binding of [^{3}H]taxol to 82H11B9F was detected. These results showed that the inhibition of 82H11B9F was caused by its taxol-mimicking character.

C. Microtubule assembly assay

Tubulin, twice purified, was used in this assay. The twice purified pellets were resuspended in MES buffer without GTP. The same volume ratio of PBS to MES buffer in radioimmunoassay was used. We found that the amount of polymerized microtubules by taxol in the PBS-MES mixture was about a half of that in pure MES buffer. In our experiments, 20 μM taxol would make an increase of 0.7 of optical density at 352 nm, with the tubulin concentration of 0.8 mg/ml.

Polymerization of tubulin occurred immediately after taxol was added to tubulin solution and arrived a peak after 15 minutes (data not shown). The sigmoid curve and time course were compatible with other reports having assembly assay in pure MES buffer **(29, 30)**. 82H11B9F at a final concentration of 6.5 μM could act as taxol to polymerize tubulin, with a 15 minute lag time to initiate turbidity change. The final peak turbidity change was about double the amount of that by 20 μM taxol. 3.25 μM of 82H11B9F needed a longer time (about 30 minutes) to start reaction and the peak change was only about two thirds of that with 6.5 μM (Figure 6). The control mixture (only PBS and tubulin, without taxol or antibodies) showed no increase of turbidity within 90 min.

Carboni et al. **(27)** reported that a C-7 substituted photoreactive taxol analog could act as taxol and polymerize tubulin. This analogue with a higher molecular weight showed a 5 minute lag time in assembly

assay. The molecular weight of an IgG molecule is about 170 times greater than a taxol molecule (Ref. Physical chemistry), and the chance that the IgG will collide with the binding sites on microtubules would be lower than for taxol. Furthermore, a larger molecule has more determinants. Therefore, in each collision, the rate of effective collision (the contact of the active part with the binding sites on microtubules) will be lower than that of a smaller molecule. These two factors, diffusion rate and effective collision, could explain the presence of a lag time for 82H11B9F in assembly assay.

D. Electron microscopy

The samples after microtubule assembly assays were checked by electron microscope with staining of 1% uranyl acetate. A large amount of microtubules were found in the grid with the 82H11B9F was identical to that induced by taxol (Fig. 6, pictures). At 400,000 x magnification, arrays of Y-shaped antibody-like molecules could be seen bound to be microtubules (Fig. 7, a picture). This finding showed that the turbidity change induced by 82H11B9F is due to microtubule polymerization, and 82H11B9F caused the same mechanism as taxol did to polymerize microtubules.

III. Discussion

Taxol, originally extracted from the bark of the western yew, Taxus brevifolia, and now made semi-synthetically, is the first of a new class of anti-cancer agents (1). It acts by promoting and irreversibly stabilizing microtubule assembly, thus interfering with the dynamic processes required for cell viability and multiplication (2). In this respect, it differs from other tubulin-reactive drugs such as colchicine, podophyllotoxin and vinblastine which inhibit tubulin polymerization (4).

The action of taxol on microtubules is not clearly understood although recent photoaffinity labeling experiments implicate b-tubulin as a site of attachment (31). With the aim of using immunological techniques to study the mechanism of action taxol, we prepared a monoclonal anti-idiotypic antibody that mimics taxol, using an auto-anti-idiotypic strategy (22,32). It inhibits the binding of [^{3}H]taxol to microtubules. Moreover, like taxol, it promotes the assembly of tubulin into microtubules.

Balb/c mice were immunized with a conjugate of BSA and taxol and hybridomas prepared as described previously for the isolation of monoclonal anti-taxol antibodies (24). In this case, however, screening by ELISA was for cell supernatants containing antibodies capable of anti-taxol antibody (24). Positive clones were subcloned twice and tested for their ability to inhibit to binding of [^{3}H]taxol to microtubules.

One antibody, 82H11B9F, an IgG_{2b}, was selected for further study because it strongly inhibited the binding of [^{3}H]taxol to microtubule preparations. It had the following characteristics. (A) Its binding to Fab' of rabbit anti-taxol was inhibited by taxol and taxotere (18), both active anti-tumor agents, but not by baccatin III, a structurally related compound devoid of anti-tumor activity (Figure 1). (B) It inhibited the binding of [^{3}H]taxol to microtubules with an IC_{50} of 1uM (Figure 2). A control monoclonal IgG2b antibody, specific for thymine glycol (28), was inactive. The reliability of the assay was tested by assaying for inhibition by taxol, taxotere and baccatin III. Baccatin III, which is structurally related to taxol but inactive, did not inhibit [^{3}H]taxol binding. Control experiments were also run to show that 82H11B9F did not bind taxol. (C) When incubated with tubulin at 37°C and followed turbidimetrically (19), it

induced the formation of microtubules which, like those induced by taxol, were not dissociated by Ca^{2+} (30). As with taxol, the curve was sigmoidal although the lag period was long (Figure 3). This is accord with the relatively high molecular weight of the immunoglobulin molecule compared to taxol, with the resulting slower rate of diffusion (33), and the lesser probability of a productive collision, i.e one in which contact is made by the combining site of the antibody, which occupies a relatively small part of the macromolecule. (D) Electron microscopic examination of the product revealed typical microtubule structures (34) with arrays of antibody-like molecules along the surface (Figure 3). The microtubules measured about 240A in diameter, some were as long as 2 um. The antibody-like molecules were about 125A in size. Both measurements are in agreement with data in the literature (34).

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What is claimed is:

1. A monoclonal antibody capable of binding to taxol or taxol-like substances produced by a hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281.

2. A monoclonal antibody capable of binding to taxol or taxol-like substances produced by a hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280.

3. The monoclonal antibody of claims 1 or 2, wherein the taxol-like substances are biologically active taxol derivatives.

4. The hybridoma cell designated 69E4A8E and having ATCC Accession No. HB 11281 which produces the monoclonal antibody of claim 1.

5. The hybridoma cell designated 29B7B3C and having ATCC Accession No. HB 11280 which produces the monoclonal antibody of claim 2.

6. The monoclonal antibody of claims 1 or 2 labeled with a detectable marker.

7. The monoclonal antibody of claim 6, wherein the detectable marker is a radioactive isotope, enzyme, dye, fluorescent marker or biotin

8. The monoclonal antibody of claim 6, wherein the detectable marker is a radioactive isotope.

9. The monoclonal antibody of claim 6, wherein the detectable marker is an enzyme.

10. A method for detecting the presence of taxol or a taxol-like substance in a sample which comprises:

a) treating the sample with a monoclonal antibody of claims 1 or 2 under conditions permitting the antibody to bind to taxol or the taxol-like substance and form a complex therewith;

b) removing antibodies which did not bind to taxol or the taxol-like substance; and

c) detecting the presence of the antibodies, thereby detecting the presence of taxol or the taxol-like substance in the sample.

11. The method of claim 10, wherein the sample is plant extracts.

12. The method of claim 10, wherein the sample is a biological fluid.

13. The method of claim 12, wherein the biological fluid is serum.

14. The method of claim 12, wherein the biological fluid is urine.

15. The method of claim 10, wherein prior to step (c) the sample is diluted approximately 1:5 with a suitable diluent.

16. The method of claim 15, wherein the sample is a biological fluid sample.

17. The method of claim 16, wherein the biological fluid is serum.

18. The method of claim 16, wherein the biological fluid is urine.

19. The method of claim 15, wherein the sample is cytosols from cells derived from animals or plants.

20. The method of claim 10, wherein step (c) comprises:

i) contacting the sample with a second antibody labeled with a detectable marker, wherein the second antibody is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance;

ii) removing any unbound antibody which is labeled with a detectable marker;

iii) detecting the presence of the detectable marker on an appropriate substrate, thereby detecting the presence of taxol or a taxol-like substance in the sample.

21. The method of claim 20, wherein the detectable marker is a radioactive isotope, enzyme, dye, fluorescent marker or biotin.

22. The method of claim 20, wherein the detectable marker a radioactive isotope.

23. The method of claim 20, wherein the detectable marker is an enzyme.

24. The method of claim 20, wherein the sample is plant extracts.

25. The method of claim 20, wherein the sample is a biological fluid.

26. The method of claim 25, wherein the biological fluid is serum.

27. The method of claim 25, wherein the biological fluid is urine.

28. The method of claim 20, wherein the sample is cytosols from cells derived from animals or plants.

29. A method for detecting the presence of taxol or a taxol-like substance in a sample which comprises:

a) contacting a predetermined amount of taxol or taxol-like substance labeled with a detectable marker with either of the monoclonal antibody of claims 1 or 2 under conditions permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance;

b) contacting the sample with the complex of step (a) such that any taxol or taxol-like substance in the sample will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody;

c) separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance; and

d) detecting the presence of any bound, detectably labeled taxol or taxol-like substance, thereby detecting the presence of taxol or taxol-like substance in the sample.

30. The method of claim 29, wherein in step (c), the

bound labeled or unlabeled taxol or taxol-like substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by addition of a charcoal solution and centrifugation.

31. The method of claim 29, wherein in step (c), the bound labeled or unlabeled taxol or taxol-like substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by precipitation of the complex comprising monoclonal antibody and the labeled or unlabeled taxol or taxol-like substance bound thereto.

32. The method of claim 31, wherein the precipitation is facilitated by addition of a sodium sulfate solution.

33. The method of claim 31, wherein the precipitation is facilitated by addition of polyethylene glycol.

34. The method of claim 29, wherein in step (c), the bound labeled or unlabeled taxol or taxol-like substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by contacting the complex formed in step (b) with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance.

35. The method of claim 29, wherein the sample is plant extracts.

36. The method of claim 29, wherein the sample is a biological fluid.

37. The method of claim 36, wherein the biological fluid is serum.

38. The method of claim 36, wherein the biological fluid is urine.

39. The method of claim 29, wherein the sample is cytosols from cells derived from animals or plants.

40. A method for screening for a ligand in a subject which is not being treated with taxol which comprises:

a) obtaining a sample from a subject;

b) contacting the sample with the monoclonal antibody of claims 1 or 2 under conditions permitting the antibody to any taxol-like substance in the sample and form a complex therewith;

c) removing any antibodies which did not bind to the taxol-like substance; and

d) detecting the presence of antibodies in the sample, thereby detecting the presence of the ligand in the subject.

41. The method of claim 40, wherein the sample is a biological fluid.

42. The method of claim 41, wherein the biological fluid is serum.

43. The method of claim 41, wherein the biological fluid is urine.

44. The method of claim 26, wherein the sample is cytosols from cells derived from the subject or from cell culture.

45. The method of claim 40, wherein step (d) comprises:

i) contacting the sample with a second antibody which is labeled with a detectable marker and is capable of binding to the antibody capable of binding to taxol or a taxol-like substance;

ii) removing any unbound antibody which is labeled with a detectable marker; and

iii) detecting the presence of the detectable marker, thereby detecting the presence of taxol or taxol-like substance in the sample.

46. The method of claim 45, wherein the detectable marker is a radioactive isotope, enzyme, dye, fluorescent marker or biotin.

47. The method of claim 45, wherein the detectable marker is a radioactive isotope.

48. The method of claim 45, wherein the detectable marker is an enzyme.

49. The method of claim 40, wherein the sample is a biological fluid.

50. The method of claim 49, wherein the biological fluid is serum.

51. The method of claim 49, wherein the biological fluid is urine.

52. The method of claim 45, wherein the sample is cytosols from cells derived from the subject.

53. A ligand recognized by the method of claim 40.

54. A ligand recognized by the method of claim 45.

55. A polypeptide recognized by the method of claim 40.

56. A polypeptide recognized by the method of claim 45.

57. A method of quantitatively determining the amount of taxol or taxol-like substance in a sample which comprises:

a) contacting a solid support with an excess of a composition of matter comprising taxol and an appropriate carrier molecule under conditions permitting the composition of matter to attach to the surface of the solid support;

b) contacting the solid support to which the composition of matter is attached with a suitable blocking agent or buffer;

c) contacting a predetermined amount of a biological fluid sample and a predetermined amount of the monoclonal antibody of claims 1 or 2, under such conditions permitting the taxol or taxol-like substance in the sample to bind to the monoclonal antibody and form a complex therewith in solution;

d) contacting the solid support to which the composition of matter is attached with the solution of step (c) under conditions permitting antibodies which do not form a complex with the taxol or taxol-like substance in the biological fluid sample to bind to the composition of matter;

e) treating the solid support so that only the

composition of matter and monoclonal antibody bound thereto remain; and

f) determining the amount of antibody bound to the composition of matter, thereby determining the concentration of taxol or taxol-like substance in the biological fluid sample.

58. The method of claim 57, wherein the sample is plant extracts.

59. The method of claim 57, wherein the sample is a biological fluid.

60. The method of claim 59, wherein the biological fluid is serum.

61. The method of claim 59, wherein the biological fluid is urine.

62. The method of claim 57, wherein the sample is cytosols from cells derived from animals or plants.

63. A method of quantitatively determining the amount of taxol or taxol-like substance in a sample which comprises:

a) contacting a predetermined amount of detectably labeled taxol or taxol-like substance with either of the monoclonal antibody of claims 1 or 2 under conditions permitting the monoclonal antibody to bind to the detectably labeled taxol or taxol-like substance;

b) contacting the sample with the complex of step (a) under appropriate conditions such that any taxol or taxol-like substance in the sample

will displace the detectably labeled taxol or taxol-like substance bound to the monoclonal antibody;

c) separating any bound, labeled or unlabeled taxol or taxol-like substance from unbound, labeled or unlabeled taxol or taxol-like substance; and

d) determining the amount of bound, detectably labeled taxol or taxol-like substance, thereby determining the amount of taxol or taxol-like substance in the sample.

64. The method of claim 63, wherein in step (c), the bound labeled or unlabeled taxol or taxol-like substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by addition of a charcoal solution and centrifugation.

65. The method of claim 63, wherein in step (c), the bound labeled or unlabeled taxol or taxol-like substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by precipitation of the complex comprising monoclonal antibody and the labeled or unlabeled taxol or taxol-like substance bound thereto.

66. The method of claim 65, wherein the precipitation is facilitated by addition of a sodium sulfate solution.

67. The method of claim 65, wherein the precipitation is facilitated by addition of polyethylene glycol.

68. The method of claim 63, wherein in step (c), the bound labeled or unlabeled taxol or taxol-like

substance is separated from the unbound labeled or unlabeled taxol or taxol-like substance by contacting the complex formed in step (b) with an antibody capable of binding to the monoclonal antibody which is capable of binding to the taxol or taxol-like substance.

69. The method of claim 63, wherein the sample is plant extracts.

70. The method of claim 63, wherein the sample is a biological fluid.

71. The method of claim 70, wherein the biological fluid is serum.

72. The method of claim 70, wherein the biological fluid is urine.

73. The method of claim 63, wherein the sample is cytosols from cells derived from animals or plants.

74. A kit for assaying for taxol or a taxol-like substance in a sample comprising in separate compartments:

a) the monoclonal antibody of claims 1 or 2;

b) a second monoclonal antibody which is capable of binding to the antibody which is capable of binding to taxol or a taxol-like substance; and

c) a standardized solution of taxol.

75. The kit of claim 74, further comprising a plate having a plurality of wells, each well coated with

a layer of a complex comprising taxol and a suitable carrier molecule.

76. The kit of claim 75, wherein the second monoclonal antibody is labeled with a detectable marker and the kit further comprises an appropriate substrate to detect the detectable marker.

77. A method for monitoring the treatment of a disease in a subject being treated with taxol or a taxol-like substance which comprises using the kit of claim 74 to determine the amount of taxol or taxol-like substance in a sample taken from the subject.

78. The method of claim 77, wherein the disease is breast cancer or ovarian cancer.

79. The method of claim 77, wherein the disease is characterized by the presence of a melanoma.

80. An anti-idiotypic monoclonal antibody of taxol or a biologically active taxol derivative.

81. A Fab' fragment of the anti-idiotypic monoclonal antibody of claim 80.

82. A $F(ab')_2$ fragment of the anti-idiotypic monoclonal antibody of claim 80.

83. The anti-idiotypic monoclonal antibody of claim 80, which binds to a receptor to which taxol or a biologically active taxol derivative binds.

84. The anti-idiotypic monoclonal antibody of claim 83 wherein the receptor is a receptor on tubulin.

85. The anti-idiotypic monoclonal antibody of claim 83

wherein the receptor is a receptor on microtubules.

86. The anti-idiotypic monoclonal antibody of claim 80, which mimics taxol and biologically active taxol derivatives by promoting the assembly of tubulin into microtubules.

87. The anti-idiotypic monoclonal antibody of claim 80 which is an IgG_{2b}.

88. A hybridoma which produces an anti-idiotypic monoclonal antibody of claim 80.

89. The hybridoma of claim 88 designated 82H11B9F and having ATCC Accession No. HB 11548.

90. The anti-idiotypic monoclonal antibody which is produced by the hybridoma of claim 89.

91. The anti-idiotypic monoclonal antibody of claim 90 labeled with a detectable marker.

92. The anti-idiotypic monoclonal antibody of claim 91 wherein the detectable marker is a radioactive isotope, enzyme, dye, fluorescent marker or biotin.

93. A method of detecting receptors which bind taxol or biologically active taxol derivatives in a sample which comprises contacting the sample with the anti-idiotypic monoclonal antibody of claim 80 under conditions permitting the antibody to form a complex with a receptor and detecting the presence of any complex so formed.

94. A qualitative immunoassay for detecting a receptor which binds taxol or biologically active taxol receptors which method comprises:

a) contacting a sample suspected of containing the taxol receptor with the anti-idiotypic monoclonal antibody of claim 91 under conditions permitting the formation of a complex which comprises the detectably labeled anti-idiotypic monoclonal antibody and the taxol receptor; and

b) detecting the presence of the complex and thereby detecting the presence of the taxol receptor.

95. The method of claims 93 or 94 wherein the sample is derived from biological fluids.

96. The method of claim 95 wherein the biological fluids comprising serum or tissue extracts.

97. A qualitative histochemical assay for detecting the presence of a taxol receptor in a biological sample which comprises:

a) contacting the biological sample with the anti-idiotypic monoclonal antibody of claim 80 under conditions permitting the formation of a complex which comprises the taxol receptor and the anti-idiotypic monoclonal antibody;

b) removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex;

c) contacting the resulting sample with a detectably labeled antibody or detectably labeled antibody fragment capable of specifically binding the anti-idiotypic monoclonal antibody under conditions permitting

the labeled antibody or antibody fragment to bind to the anti-idiotypic monoclonal antibody; and

d) determining the presence of detectably labeled antibody fragment bound to the anti-idiotypic monoclonal antibody and, thereby, detecting the presence of taxol receptor in the sample.

98. A quantitative histochemical assay for determining the amount of a taxol receptor present in a biological sample which comprises:

a) treating the biological sample with a predetermined amount of the anti-idiotypic monoclonal antibody of claim 80 under conditions permitting the formation of a complex which comprises the taxol receptor and the anti-idiotypic monoclonal antibody;

b) removing from the sample anti-idiotypic monoclonal antibody which is not part of the complex;

c) contacting the resulting sample with a detectably labeled antibody or a detectably labeled antibody fragment capable of specifically binding to the anti-idiotypic monoclonal antibody under conditions permitting the detectably labeled antibody or detectably labeled antibody fragment to bind to the anti-idiotypic monoclonal antibody which is part of the complex formed in (a); and

d) determining the amount of detectably labeled antibody or detectably labeled antibody fragment bound to the anti-idiotypic monoclonal

antibody and thereby determining the amount of taxol receptor in the sample.

99. A method for determining the amount of taxol or biologically active taxol derivative in a sample which comprises:

a) immobilizing the anti-idiotypic monoclonal antibody of claim 80 on an appropriate substrate;

b) contacting the sample containing taxol or biologically active taxol derivative with the immobilized anti-idiotypic monoclonal antibody of step (a) under conditions permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized anti-idiotypic monoclonal antibody;

c) contacting the complex formed in step (b) with a predetermined amount of a labeled antibody or labeled antibody fragment which specifically binds to taxol or biologically active taxol derivative under conditions permitting the labeled antibody or labeled antibody fragment to displace taxol or biologically active taxol derivative bound to the immobilized anti-idiotypic monoclonal antibody; and

d) determining the amount of labeled antibody or labeled antibody fragment bound to the immobilized monoclonal anti-idiotypic monoclonal antibody thereby determining the amount of taxol or biologically active taxol in the sample.

100. A method for determining the amount of taxol or

biologically active taxol derivative in a sample which comprises:

a) immobilizing an antibody or antibody fragment which specifically binds to taxol or biologically active taxol derivatives on an appropriate substrate;

b) contacting the sample containing taxol or biologically active taxol derivative with the immobilized antibody or antibody fragment of step (a) under conditions permitting any taxol or biologically active taxol derivative in the sample to form a complex with the immobilized antibody or antibody fragment;

c) contacting the complex formed in step (b) with a predetermined amount of the anti-idiotypic monoclonal antibody of claim 80 labeled with a detectable marker under conditions permitting the labeled anti-idiotypic monoclonal antibody to displace any taxol or biologically active taxol derivative bound to the immobilized antibody or antibody fragment; and

d) determining the amount of labeled anti-idiotypic monoclonal antibody bound to the immobilized antibody or antibody fragment thereby determining the amount of taxol or biologically active taxol derivative in the sample.

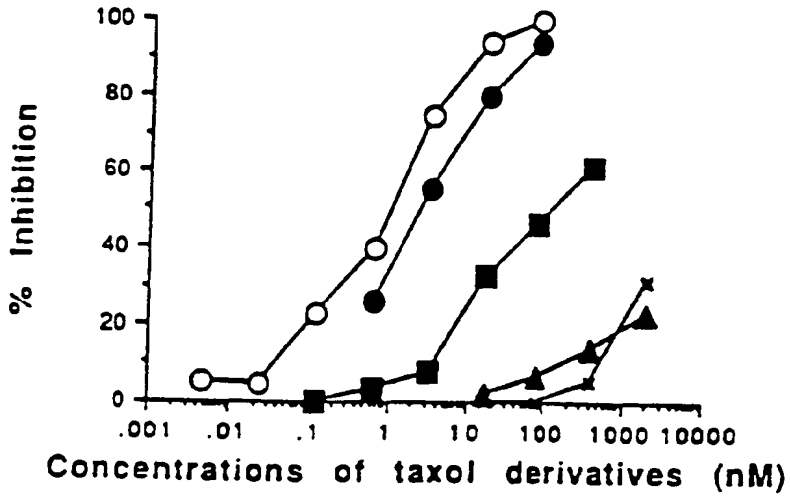
101. The method of claim 99, wherein the labeled antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281 labeled with a detectable marker.

102. The method of claim 99, wherein the labeled antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280 labeled with a detectable marker.

103. The method of claim 100, wherein the antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 69E4A8E ATCC No. HB 11281.

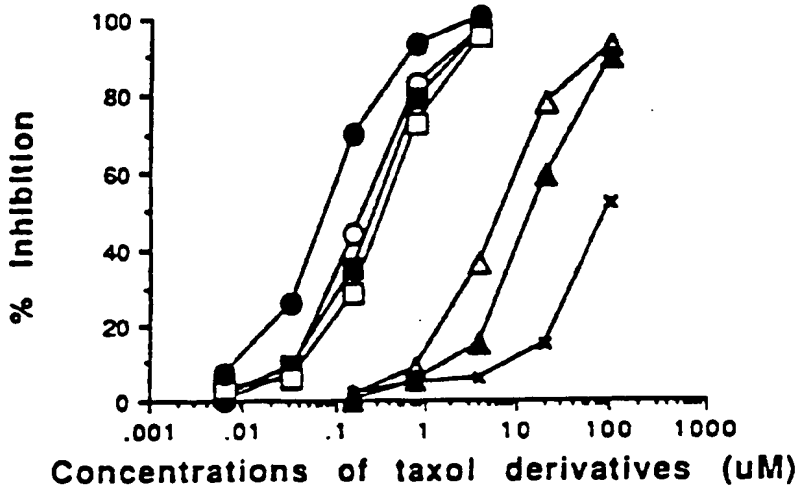
104. The method of claim 100, wherein the antibody which specifically binds taxol or biologically active taxol derivatives is the monoclonal antibody produced by the hybridoma designated 29B7B3C having ATCC Accession No. HB 11280.

1/10
FIGURE 1

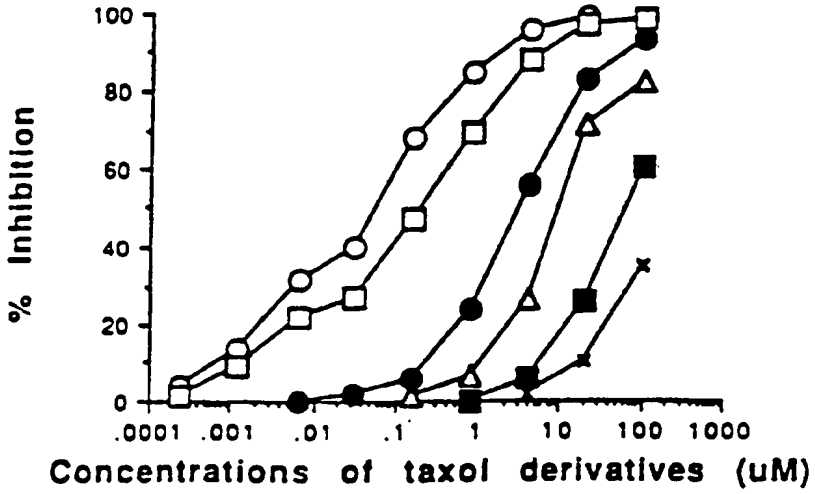

100
80
60
40
20
0
% Inhibition
.001
.01
.1
1
10
100
1000
10000
Concentrations of taxol derivatives (nM)

2/10

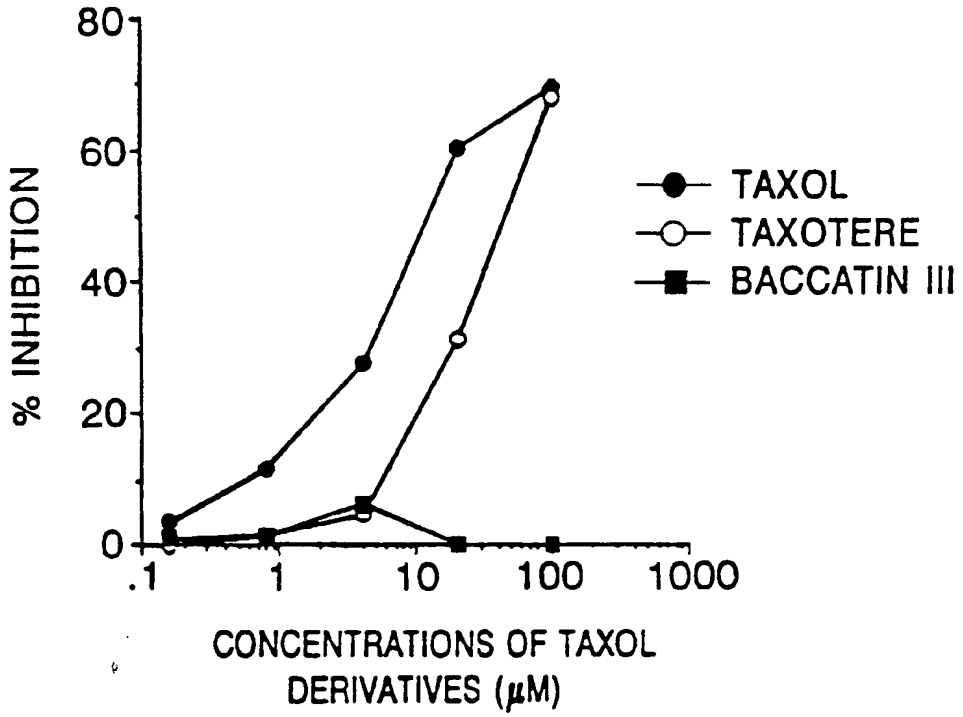
FIGURE 2


% Inhibition
100
80
60
40
20
0
.001
.01
.1
1
10
100
1000
Concentrations of taxol derivatives (uM)

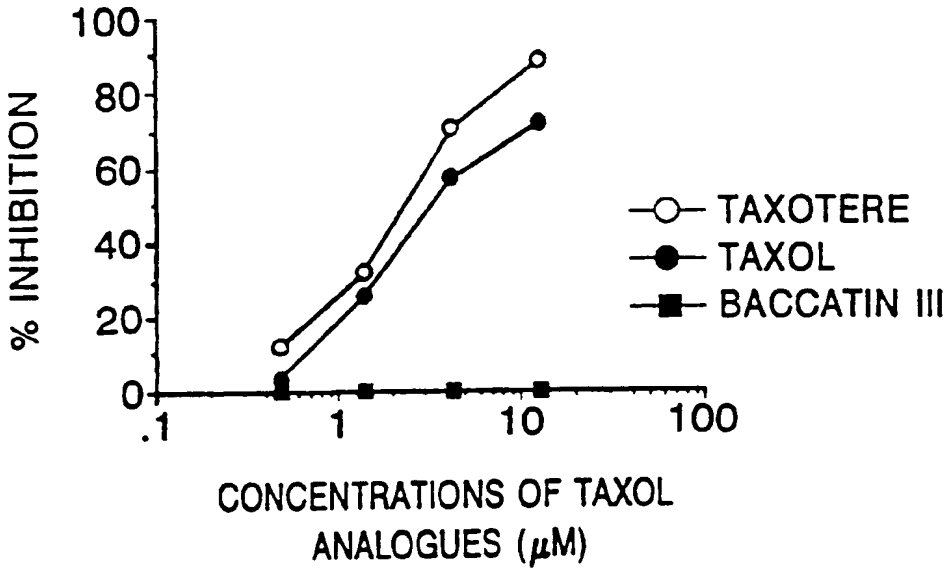
3/10
FIGURE 3


% Inhibition
100
80
60
40
20
0
.0001 .001 .01 .1 1 10 100 1000
Concentrations of taxol derivatives (uM)

4/10
FIGURE 4


80
60
40
20
0
% INHIBITION
.1
1
10
100
1000
CONCENTRATIONS OF TAXOL DERIVATIVES (μM)
TAXOL
TAXOTERE
BACCATIN III

5/10
FIGURE 5A



6/10

FIGURE 5B

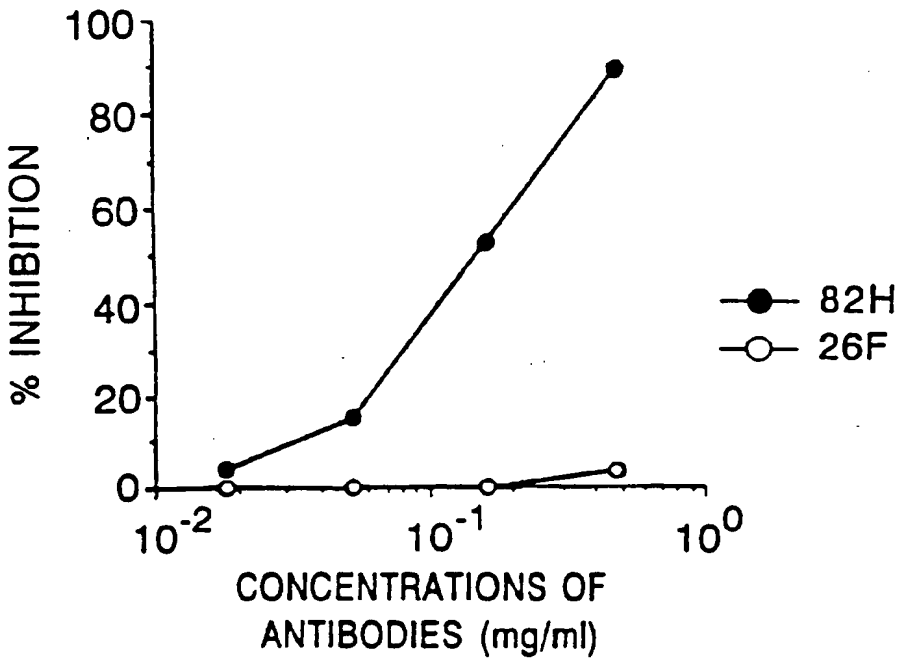


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FIGURE 6

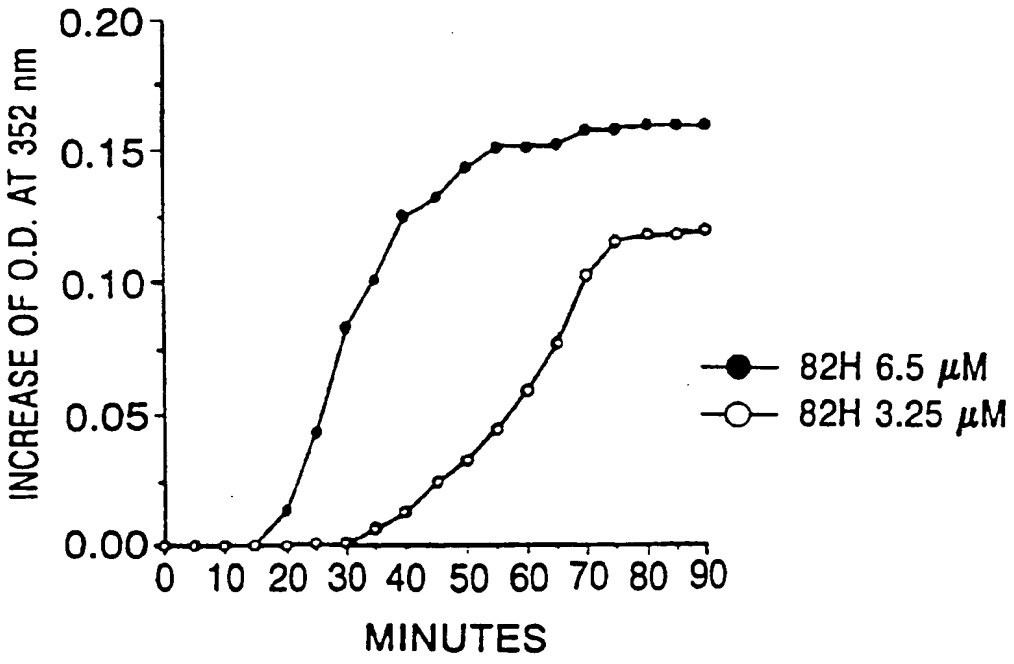
0.20
0.15
0.10
0.05
0.00
INCREASE OF O.D. AT 352 nm
0 10 20 30 40 50 60 70 80 90
MINUTES
82H 6.5 μM
82H 3.25 μM

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FIGURE 7A

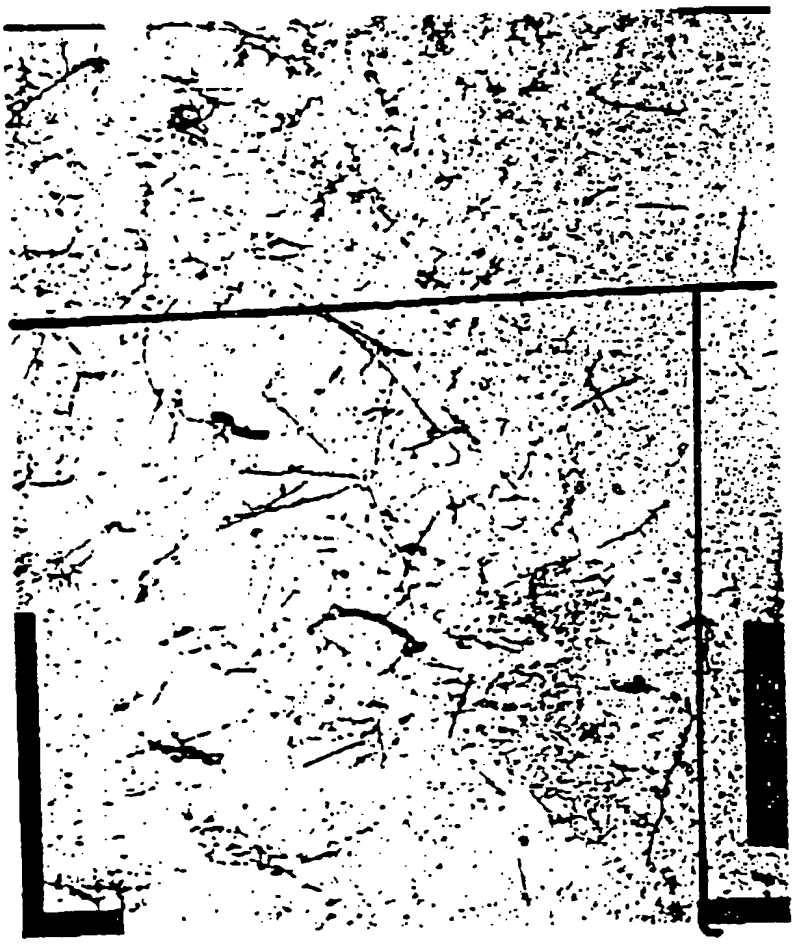

FIGURE 7B

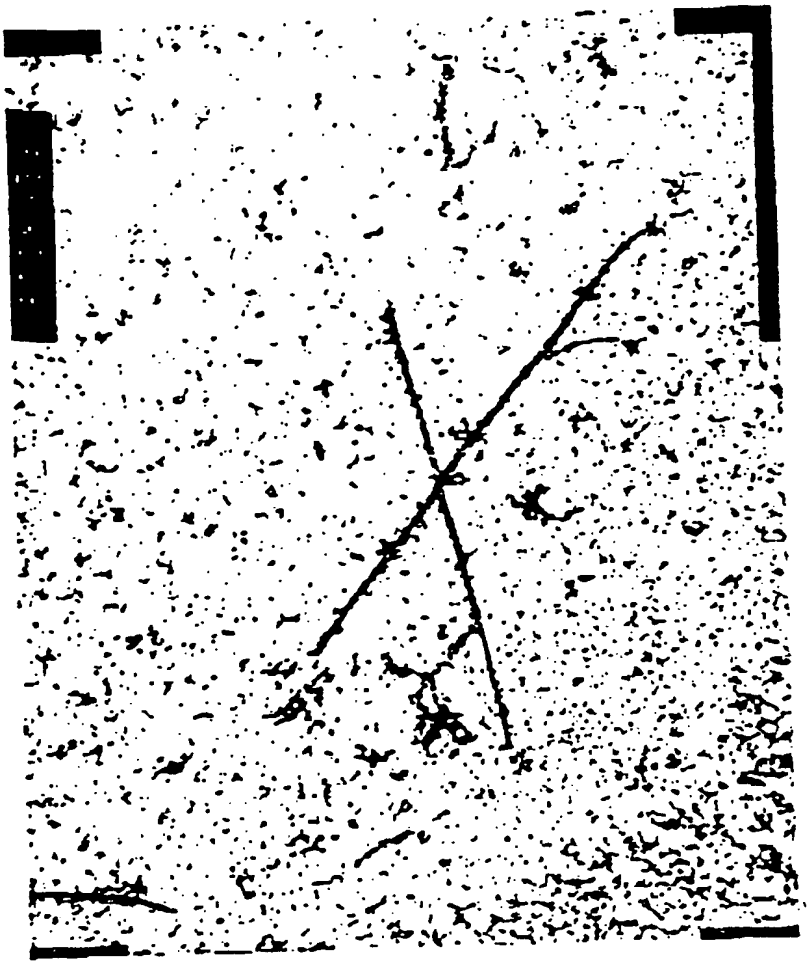

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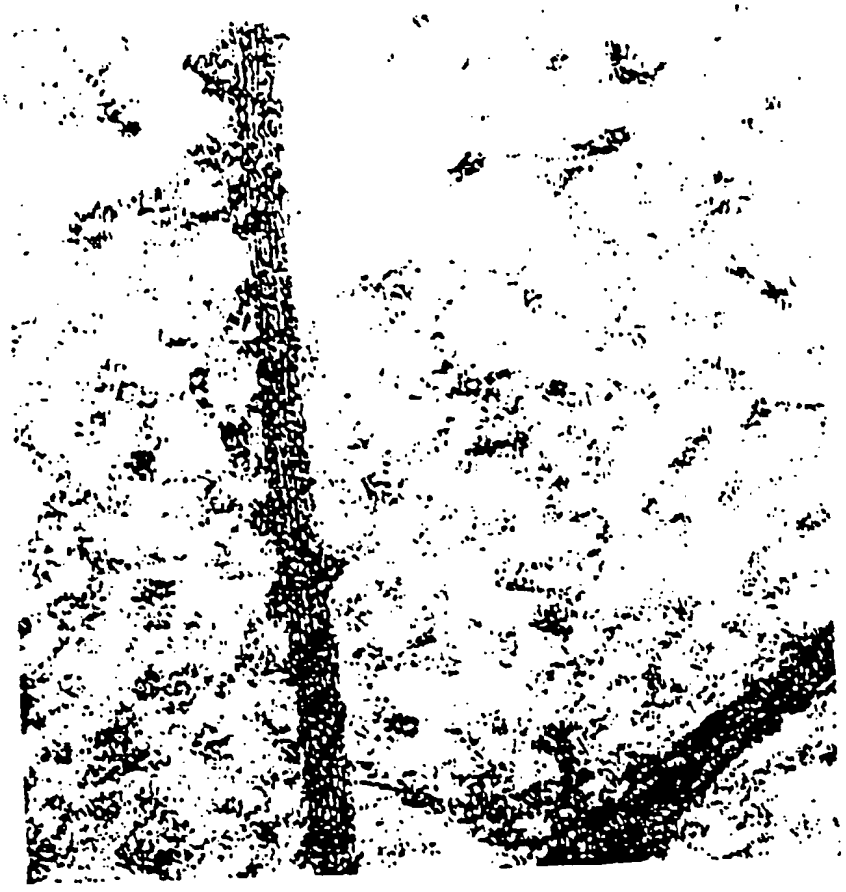
FIGURE 7C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02330

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.21, 7.9, 7.92, 7.94, 7.95, 7.95, 70.21, 240.27; 530/387.1, 387.2, 388.1, 388.25, 388.5, 391.1, 391.3, 514/449; 549/510

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Immunological Methods, Volume 158, No. 1, issued 18 January 1993, P.G. Grothaus et al. "An Enzyme Immunoassay for the Determination of Taxol and Taxanes in *Taxus* sp. Tissues and Human Plasma", pages 5-15, especially pages 5-6, 8-9, 11-12, Table I.	1-104
Y	Clinical Chemistry, Volume 27, No. 11, issued 1981, E.D. Sevier et al., "Monoclonal Antibodies in Clinical Immunology", pages 1797-1806, especially pages 1797-1799.	1-7, 9-13, 15-17, 19-26, 28, 44, 57-60, 74, 75-78, 99-100
Y	Pharmacology and Therapeutics, Volume 52, issued 1991, D.G.I. Kingston, "The Chemistry of Taxol", pages 1-34, especially page 26.	14, 18, 27, 38, 43, 51, 61, 72

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 MAY 1994	JUN 09 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	EVE J. WILSON Jill Warden for
Facsimile No. (703) 305-3230	Telephone No. 703-308-4244

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02330

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.P. Ransom, "Practical Competitive Binding Assay Methods", published 1976 by The C.V. Mosby Co. (St. Louis), Chapter 1 pages 1-9, Chapter 5 pages 54-61, Chapter 7 pages 76-113, especially pages 2-4 and 55-57.	8, 22, 29-32, 34-39, 40-52, 63-66, 68-73
Y	W.D. Odell et al., "Principles of Competitive Protein-Binding Assays", published 1983 by John Wiley and Sons, Inc. (New York), pages 107-124, especially page 115.	33, 67
Y	US, A, 4,960,790 (STELLA ET AL.), 02 October 1990, column 1, lines 10-15.	79
Y	US, A, 4,818,684 (EDELMAN ET AL.) 04 April 1989, columns 1-4; column 7, lines 19-64; column 13, line 64 to Column 14 line 45.	80-83, 88-98
Y	US, A, 5,144,010 (ERLANGER ET AL.), 01 September 1992, column 5, lines 9-11; column 61, lines 35-40 and 42-45.	84-86
Y	Journal of Cell Biology, Volume 91, issued November 1981, J. Parness et al. "Taxol Binds to Polymerized Tubulin In Vitro", pages 479-487, especially pages 482 and 483.	84-85
Y	US, A, 4,536,479 (VANDER-MALLIE) 20 August 1985, column 2, line 64 to column 3 line 15; column 5, line 64 to column 6, line 35; column 7, line 1 to column 8, line 25.	99-104
Y	Journal of Pharmacology Belgium, Volume 46, No. 2, issued 1991, M. Jaziri et al., "Enzyme-Linked Immunosorbent Assay for the Detection and the Semi-Quantitative Determination of Taxane Diterpinoids Related to Taxol in Taxus sp. and Tissue Cultures", pages 93-99, especially pages 94 and 96.	1-7,9-12, 15-16, 19-21, 23-25, 28,57-59, 62, 74-76, 99-100

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02330

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Telephone Practice

I. Claims 1-52, 57-104, drawn to monoclonal antibodies, anti-idiotypic monoclonal antibodies, and methods and kits using these monoclonal antibodies.

II. Claims 53-56, drawn to ligands and polypeptides.

Groups I and II are drawn to materially different compositions. The composition of Group II (claims 53-56) is not required to obtain the compositions of Group I and is not used in any assay of Group I. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US94/02330

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K39/00, 385, 395, 44; A61K 31/335; C07D 305/14; C07K 15/00; G01N 33/48, 53, 536, 537, 539, 541, 543, 563, 577

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.21, 7.9, 7.92, 7.94, 7.95, 7.95, 70.21, 240.27; 530/387.1, 387.2, 388.1, 388.25, 388.5, 391.1, 391.3; 514/449; 549/510

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG, STN
Search terms: taxol, cephalomannine, benzenepropanoic acid, paclitaxel, docetaxol, 7-epitaxal, taxane, CA registry numbers for these substances; antibody, anti-idiotypic antibody, endogenous, serum, sera, blood, plasma, urine, cytosol.